STUDIES OF CERTAIN METABOLITES IN RELATION TO GROWTH AND REPRODUCTION IN *DYSDERCUS CINGULATUS* F. (HEMIPTERA : PYRRHOCORIDAE)

ABSTRACT

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SHAMIM SIFAT

Department of Zoology
Aligarh Muslim University
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* [illegible] IN RELATION TO GROWTH AND REPRODUCTION IN [illegible] *

ABSTRACT

The proteins, carbohydrates and the lipids are the chief metabolites providing building material for the tissue as well as these are reserve sources of energy required at various stages of development of the organism. In various insects, although the metabolism of each of these substances has been studied in relation to the biological activity of the species, the relative changes in the synthesis of these substances were not known to be correlated with the developmental stages, age, moulting and reproduction. Therefore, in Dysdercus cingulatus F. the changes in the concentration of the total proteins (Pro.), reducing sugars (RS) and fats (FA.) in the whole body of the nymphs (4th and 5th instar) and the adults (both sexes) in relation to age, moulting and reproduction have been studied. Further, the changes in the concentration of the proteins and the reducing sugars in the ovaries have also been studied in relation to their growth and maturation.

Since reducing sugars are the intermediary substances in the carbohydrate metabolism, therefore, the changes in the concentration of total reducing sugars of the body as well as in the ovaries of D. cingulatus have been relied on to indicate the changes in the carbohydrate synthesis.

The data on _A. sinuatus_ also compare with those of _Locusta migratoria_ _manilensis_ (Norris, 1954 and 1959), _Locusta_ species (Khan, 1960), _Oxya_ _velox_ (Devonbock and Indira, 1971) and _Melanoplus_ _americanus_ (Matthews and Tanner, 1974).

In _A. sinuatus_, the sexual growth is characterized by the addition of the body weight on a large scale of the synthesis of the metabolites as evident by the changes in the concentration of the TNA, RNA and DNA in the newly moulted 4th instar nymphs and in the older nymphs. The nymphs remaining intermediate, are ten times heavier than that of the newly moulted 4th instar. However, there is significant loss of weight during moulting or metamorphosis due to the shedding of the old cuticle and the utilization of the body metabolites as usually reducing sugars and fats for obtaining energy. Thus, the pace of changes in the body weight of the nymphs related with the cuticle throws a new light on the theory of _A. sinuatus_.

In the females, the bulk of the body weight is added due to massive growth of the ovaries in contrast to the insignificant increase in the body weight of the males due to comparatively little growth in the testes. Further, in the females changes in the body weight are related with the maturation and oviposition of the eggs of different batches.

Further, the changes in the body weight are parallel to that of the DNA but inversely proportional to that of the RNS of the body of the growing nymphs of the 4th and the 5th instars.

The synthesis of both the substances are initially rapid and then gradual in each instar. Further moulting has a depleting effect in the concentration of these substances. As compared to the DNA, the RNA level is more decreased during oogyle because the latter is the chief source to provide energy by its oxidation during the oogyle. But prior to metamorphosis, the pupae accumulate almost double amount of the total proteins as compared to that of the newly moulted 4th instar nymphs.

The concentration of total proteins of the whole body of the males and the females at the time of emergence is almost similar. Later, the females significantly build up higher amount of the proteins than that of the males following emergence. Further, maximum concentration occurs prior to the oviposition of the eggs of the respective batches. Correspondingly the proteins of the ovaries are also maximum but following oviposition, the total proteins of the body as well as that of the ovaries fall down and later again synthesis follows. Although the RNA level is not very high, the changes in its concentration also follow a parallel course with the DNA. On the other hand in the males, the changes both in the DNA and the RNA are slow and insignificant which do not run parallel with that of the females.

The peak of the DNA in the ovaries prior to the oviposition is further supported by the maximum concentration of the proteins qualitatively separated by the electrophoretic method, although the number of these proteins is highest in the immature ovaries. Further, again causes depletion in the synthesis of the ovarian

proteins. On topical treatment of the last stage nymphs by LD_{50} Lindane and Parathion, the concentration of different proteins of the maturing ovarion diluted. Further fragmentation and disintegration also takes place especially by the effect of Parathion.

Following the metamorphosis, the females have lower level of the T.R.C than that of the males. It appears that the female nymphs utilise higher amount of reducing sugars to obtain energy for moulting than the males. In adults, variations in the T.R.C level are corresponding with that of the T.R.C following emergence. There is an increase in the T.R.C level up to 3rd post-emergent day when the males are sexually active. Thereafter, the decrease in the T.R.C level in the body of the males is due to their oxidative utilisation to release the energy for motility of sperms.

The changes in the metabolism of the fats are unlike those of the total proteins and the reducing sugars. In the newly moulted 4th instar nymphs the concentration of the total fats (T.F.C) of the body is maximum for the instar. But, later, it continuously falls and prior to the next moulting the T.F.C level is significantly lower than that of the youngest nymphs. On the contrary, in the final (5th instar) instar, there is progressive synthesis of the body lipids and its maximal level occurs in the fully grown nymphs (ready to metamorphosis). However, the bulk of the total lipids is added during the first half of the 5th instar. Thus, there is intensive metabolism of the fats as larval reserve for the future adults especially for the females. But

there is considerable loss in the larval fats during the metamorphosis due to its utilization in energy release for the exhaustive phenomenon of ecdysis. Against the level of the TRS, that of the TAG is higher in the newly emerged females as compared to that of the males. Later, the fall in the TAG of the males is steadily pronounced in relation to age and thus in the 12 day old males almost 1/4th concentration of the TAG of the newly emerged males is retained. On the other hand in the females, most of the larval fat is utilized in the maturation of the first batch of the eggs and a significant drop in the TAG following the oviposition occurs. However, for the maturation of the successive batches of the eggs, further synthesis of fats takes place. Therefore, unlike the males the synthesis of the fats in the females is not completely stopped due to ageing.

Thus, in *D. cingulatus* the synthesis and utilization of the total proteins, reducing sugars and the lipids vary during the developmental stages and the growth of the adults. Further, the females differ from that of the males with regard to significant changes in the metabolism of these substances related with the maturation and oviposition of the eggs. However, in both the sexes, ageing causes a decrease in the synthesis of these metabolites indicating lowering of the metabolic potential in the old age. The present observations add to our knowledge on the changes in the metabolism of the proteins, reducing sugars and fats related with development, moulting, age and reproduction on

D. cingulatus, a species which is one of the major pests of cotton crop. Moreover, the effects of lindane and Parathion on the changes in the ovarian proteins of D. cingulatus contribute to explain the mode of action of these insecticides on the metabolism of ovarian proteins of the insects in general and D. cingulatus in particular.

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This is to certify that the present thesis entitled "Studies of certain metabolites in relation to growth and reproduction in Dysdercus cingulatus F. (Heteroptera:Pyrrhocoridae)" is entirely based on the investigations made by Miss Shamim Bifat. It contains some original and important information on the metabolic activity related with growth and reproduction of insects in general and D. cingulatus F. in particular. Therefore, I permitted her to submit this thesis for the award of Ph.D. degree of Aligarh Muslim University, Aligarh.

Mumtaz A Khan

(Mumtaz A. Khan)
M.Sc. (Alig.), Ph.D. (Lond.), D.I.C.,
F.R.E.S., F.Z.S.I.
Reader in Zoology
Aligarh Muslim University, Aligarh.

Dated: MAY 24th, 1977.

C O N T E N T S

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Shamim Sifat

(Shamim Sifat)

INTRODUCTION

In insects, proteins, fats and carbohydrates provide most of the building material and chief sources of energy. The metabolism of these substances takes place almost continuously throughout the life in response to various physiological events such as growth, moulting, metamorphosis, flight and reproduction etc., and varies according to their requirements of different stages of life.

Proteins are considered as the primary component of the living matter and thus these are more important than other substances. Furthermore, proteins are especially involved in the process of growth and reproduction which are regulated by the formation and degradation of proteins as well as nucleic acids in the presence of enzymes that are basically proteins. It is also known that insect hormones are directly or indirectly linked to protein metabolism. Burdette and Coda (1963) reported that ecdysone i.e. the moulting hormone of the prothoracic glands of insects, even enhances the synthesis of hepatic proteins in mammals.

Generally, the protein synthesis is high during the growth of insects and particularly at the time of metamorphosis. In *Bombyx mori*, protein synthesis is greatly increased to produce

silk. However, the data on the protein metabolism appear to be very little in relation to reproductive activity of insects. It is, therefore, essential to study this aspect in a variety of insects. With this view, in Dysdercus cingulatus Fabr. changes in total proteins of the whole body have been recorded during the 4th and the 5th instars as well as in the adults in relation to two reproductive cycles which occur in about 12 days following emergence at the present controlled temperature and humidity. Further, protein concentration was also quantitatively estimated in the ovaries during maturation, oviposition and postoviposition periods. Since very little is known about various proteins of the ovaries, in this species different proteins of the ovaries were separated in relation to the age of the normal adults as well as in that of the treated females by insecticides (Lindane and Parathion).

Although the metabolism of carbohydrates in insects takes place almost continuously throughout the larval life, during the metamorphic changes there is generally depletion in the carbohydrate reserve. Among these, the disaccharide of glucose (α-D-glucopyranosyl- α-D-glucopyranoside), trehalose (a predominant sugar of insect haemolymph) has been recognised as the chief source of sugar in insects (Wyatt, 1967). The occurrence of monosaccharides (reducing sugars) in insects haemolymph is generally exceptional. But in the hymenopterous insects the level of the reducing sugars is very high (Czarnovsky, 1954 and Fischl and Ishay, 1971). Since glucose is

an important reducing sugar which is utilised in the oxidative metabolism to get energy as well as it plays a control role in carbohydrate metabolism, it is thus important to study the changes in the glucose metabolism in the whole body in relation to the developmental stages. However, such investigation has generally been neglected. Therefore, in the present project on D. cingulatus changes in total reducing sugars concentration have been studied in relation to larval development, metamorphosis and reproduction.

Fats serve as chief form to store energy and it is generally present in highest concentration in the mature larva before metamorphosis. Along with the metabolism of carbohydrates, fats provide the bulk of energy in insects. The various types of fats and their concentration in the body has been studied in a variety of insects in different physiological conditions of insect life (Wigglesworth, 1972). Although changes in total fat concentration were recorded in relation to larval growth and metamorphosis in several insects, similar investigation is almost negligible in heteropterous pests. Therefore, in D. cingulatus, total fat concentration of the body has been studied in relation to larval growth, metamorphosis and reproduction.

Dysdercus cingulatus Fabr. commonly known as red cotton bug is an important pest of cotton in Uttar Pradesh, Bihar, Madhya Pradesh, Bombay, Madras and Punjab (Sohi, 1964). It also feeds on other malvaceous plants, wheat, maize, bajra and

certain other crops of economic value. Both the nymphs and the adults feed gregariously on the leaves and bolls of cotton plants. The yellow excreta of the bug stains the lint and spoil its quality.

The present observations on the changes in total proteins, reducing sugars and fats of the body in relation to growth, moulting, metamorphosis and reproduction of D. cingulatus would form a part of fundamental knowledge in the control of this species. Further, the information on the changes in the protein pattern of the ovaries of females treated with Lindane and Parathion throws light on the action of these chemicals on the protein metabolism of the ovaries. This information may also be utilized in controlling the fecundity of this pest.

In the following section a review of the information on the proposed aspects of the present investigation has been given.

I. REVIEW

Many investigators have studied the metabolic changes during growth, development and metamorphosis in various species of insects. The information on different aspects of metabolism of various constituents of the tissues were reviewed by Gilmour (1961 and 1965), Chen (1966), Wyatt (1961 and 1967), Mast (1964), Gilby (1965), Gilbert (1967) and Wigglesworth (1972).

The present text only deals with the available information on the changes in the concentration of total proteins, fats and reducing sugars as well as the effect of poisons on different proteins of the whole body of insects in different developmental stages as well as on those of maturing ovaries.

PROTEIN METABOLISM

The crude protein content in the whole body of the nymphs and the adults was studied in various orthopteroid insects e.g. in Melanoplus species (Mc Hargue, 1917), Oxya species (Korigawa, 1934 and Ichikawa, 1937), Nomadacris septemfasciata (Rosedale, 1945), Schistocerca gregaria (Das, 1945) and Zonocerus species (Massieu and Cravioto et al., 1959). In these species, the changes in the concentration of crude proteins were related to the stages of growth. Ichikawa (1937) reported that the relative percentage of nitrogen compounds in

the two sexes of Oxya species varied due to the age and not due to the sexual difference.

Chen (1952) observed the changes in total protein concentration in the whole body of adult Locusta migratoria migratoricides and found that mean nitrogen content had some changes during adult life, but these were not very prominent. However, the protein content of a female locust reached its maximum just before oviposition.

By disc electrophoresis on polyacrylamide gel analysis of the saline extract from matured ovaries of Leucophaea maderae showed an increase in the relative concentration of five detected bands which were presumed to be yolk (Dejmal and Breuker, 1968). In the same species, Schaurer (1969) reported the occurrence of more soluble proteins in the ovaries than in the haemolymph and in the former tissue their number increased from 15 to 21 during oocyte maturation, suggesting the synthesis of some proteins in the oocytes or in the follicle cells. At the same time there was evidence for some proteins becoming insoluble in the course of yolk formation.

Among coleopterous insects, Evans (1934) recorded a gradual decrease in soluble and insoluble proteins of the whole body of Tenebrio molitor on 7th and 9th day respectively from the initiation of metamorphosis. The insoluble proteins again increased before the emergence of adult beetle, while soluble proteins decreased.

In Tenillia laevigata, the nitrogen present in total proteins of the body significantly decreased between larval and early pupal stage, remained constant in the late prepupa, but increased to a maximum percentage of the total nitrogen on the 2nd and 3rd day of the pupal period (Anderson, 1940). By the late pupal period it returned to its larval level and maintained this through emergence.

In Tribolium confusum, synthesis of proteins generally decreased with age of the larvae upto the prepupal stage, followed by an increase in the midpupal stage and again declined on adult emergence (Srivastava and Chaudhary, 1969). According to Kannavar and Nair (1969), protein concentration enormously increased in the diapausing larva of Trogoderma granarium, which only decreased after the mobilization to energy demands during prolonged diapause.

Mahota (1970) studied the changes in the soluble ovarian proteins in the bark beetle, Dendroctonus pseudotsugae in relation to ovarian development and recorded a decline in the total protein concentration in the ovaries in about 48 hours after the attack on the host logs. Further, by electrophoretic method, he observed two comparatively prominent protein fractions which did not change up to 48 hours of ovarian development. After 72 hours, two more distinct protein fractions appeared, which constituted about 49% of the total soluble proteins in the ovaries. Finally at the end of ovarian maturation (4 days), the last two fractions formed about 61% of the

total soluble proteins of the ovaries.

In lepidopterous insects, protein metabolism was studied as early as 1884 and 1887 by Kellner et al. in the larval stages as well as in the adult Bombyx mori. According to them, about half the proteins of the fully developed silkworms was used to make silk. They recorded a fall in the relative amount of protein nitrogen at the pupal stage, which was followed by a marked rise in adults.

Heller (1926) made a detailed study on the protein metabolism in the hawk moth, Celerio euphorbiae in order to compare with that of Bombyx mori. During the formation of pupa, hawk moth lost one quarter of its proteins as compared to nearly two third (2/3) in Bombyx mori. Thus, during the pupal period C. euphorbiae used larger proportion of proteins than that in B. mori.

By disc electrophoresis, proteins were studied in different tissues including the ovaries during the developmental stages of cecropia silkworm, Hyalophora cecropia (Patel, 1971). Among all the tissues studied, the ovaries and the eggs had lower capacity to synthesize proteins and not more than 8 to 12 protein fractions were detectable in the young ovaries. The number of these fractions decreased with the development of the ovaries.

In the oocytes of Ephestia kuhniella, proteins were synthesized in the yolk. But in the fully matured ovaries,

the follicle cells were found to be the most active site of protein synthesis. However, in this species stage-specific pattern of proteins were demonstrated in developing stages following the larval pupal ecdysis (Imberski and Gerson, 1974).

Among dipterous insects, in Lucilia sericata, soluble proteins slightly increased during the larval growth, but in the prepupa these proteins increased at the expense of the decreasing insoluble proteins which were being utilized for the formation of hard puparium (Bruns, 1932). On the contrary, Rainey (1950) observed that there was no greater change in the total protein composition of the body during first two larval instars of L. sericata, but in the subsequent instars accumulation of large bulk of proteins took place. Further, in this species, Lillian and Hirt (1972) studied the quantitative significance of protein synthesis during metamorphosis. It was calculated that the rate of protein synthesis was high in the pupa and in early adult development. Further, high rate of protein synthesis by head, thorax and abdomen were maintained during the period shortly before and after adult emergence. They concluded that formation of the proteins for adult growth occurred by its synthesis during the metamorphosis. Further, changes in the dry weight and the protein content of the body during diapause and normal development of Lucilia sericata was studied by King (1973). It was found that during the normal development proteins per dry weight level markedly fluctuated during the larval and the pupal stages, and increased levels

were correlated with the formation of the new cuticle etc. prior to ecdysis as well as histogenesis of the adult tissues. The protein level remained relatively high and constant during the adult life. But during diapause of the larvae, protein/dry weight level maintained at a low level, reflecting the minimal metabolic activity during this period.

Church and Robertson (1966) analysed the changes in wet and dry weight as well as total proteins and its fractions in Drosophila melanogaster from hatching of the egg to the adult. The rate of increase of total proteins was high until about 36 hours after hatching then it rapidly declined during the rest of the larval life. Both the insoluble proteins and the chitin nitrogen reached to a maximum level (about 70% total nitrogen) in the late pupa when the soluble nitrogen had its lowest level. Further, the amino acid fractions reached to a maximum level in the early third instar. In Drosophila subobscura, the rate of protein synthesis was higher in the aged flies than in the young ones (Clark and Maynard, 1966). However, in the males, the testis did not contribute a major part of total proteins and the rate of the protein synthesis was about twice as great in old as in young flies. In Musca vicina, although there was an apparent high protein concentration in the older flies than in the young ones, the rate of protein synthesis was higher in younger flies than that of the older ones (Levenbook and Indira, 1971). The cause of apparent high protein content in old flies was attributed to lower

turnover of precursor pool. Similar data were recorded in D. melanogaster (Baumann and Chen, 1959).

The changes in the wet weight and the protein content in the ovaries of wild type strain of D. melanogaster were studied from emergence to 7 days (Cummings et al., 1971). It was recorded that both the wet weight and the protein content rapidly increased from day 1 to day 4 and reached a maximum level in 6 day old flies. This increase was also correlated with the appearance of vitellogenic, post-vitellogenic and of oogenesis stages. In the yolk proteins of D. melanogaster, D. virilis and D. silvestris, 1-2 predominant proteins were found in the crude extracts of the matured oocytes and in the haemolymph of the matured females, which were synthesised in the fat body (Gelti-Bonin et al., 1974). The formation and the accumulation of the late stage gametes in the testes of Drosophila hydei during maturation was accompanied by 4-fold increase in the rate of protein synthesis (Geer et al., 1972).

In the last stage larvae of Calliphora stygia, the total body proteins remained constant in 5 to 7 day old larvae, but later, it increased up to eleventh day (Martin et al., 1969). In Musca domestica, variations in the haemolymph proteins during a gonotrophic cycle and after poisoning with some pesticides and X-ray irradiation were studied by the use of polyacrylamide gel electrophoresis (Rasnade, 1967 and Le Bras et al., 1973). They detected 14 major proteins in both sexes, but the

intensity of these fractions varied according to sex, age and feeding state. The application of γ-BHC on the pupae induced very marked decrease in the haemolymph proteins in the adults surviving poisoning. Sublethal dose of γ-BHC (LD_{40}) more selectively inhibited synthesis and accumulation of the female sex protein fraction.

In the ovaries of Diptera dorsetica, there was 6-fold increase in the total proteins content during the maturation of the ovaries and the first egg cycle (Gadallah et al., 1970a). After egg deposition, there was a cyclic decrease and increase in the protein concentration during the development of the eggs of the second cycle. A predominant protein bound with lipid and carbohydrate was present in the vitellogenic females and absent in the males of Aedes aegypti (Hagedorn and Judson, 1972).

In Culex pipiens molestus the protein spectrum for each developmental stage was specific i.e. 14 fractions were found in the eggs whereas 10 fractions in the larvae, the pupae and the adults irrespective of sex (Paulov and Paulovová, 1973). The position and concentration of single component was not equal.

The protein metabolism in relation to growth and maturation was studied in only a few hemipterous insects and most of the information deals with the studies in the haemolymph and the fat body of the larval as well as the adult forms. However, in Dysdercus cingulatus during the first gonotrophic cycle changes in total ovarian proteins appeared to be controlled

independently of the protein built up in the blood (Prabhu and Nayer, 1971).

The newly emerged female of the periodical cicada, Magicicada cassini contained about twice (110 mg) soluble proteins than that of the males (51 mg) of the corresponding age and during 13 day period, the females lost a significant amount of the soluble proteins to maintain the egg production during the adult stage (Brown and Chippendale, 1973).

METABOLISM OF REDUCING SUGAR:

In Anisolabis littorea (Reader and Bedford, 1972) and Blaberellia annulipes (Veera and Rao, 1976) moderate amount of glucose was found in the whole body as well as in the blood. However, in the latter species, both the males and the females had glucose as the principal sugar in the haemolymph as well as in different tissues. Ma et al. (1970) studied glucose metabolism in the male and the female cockroaches and found that pentose cycle was more active in the male Periplaneta americana than that of the female. Determination of glucose metabolism was made in relation to the metabolic regulation in the flight muscles of Locusta migratoria (Bucher and Klingenberg, 1958).

In the adult bee, Apis mellifica concentration of glucose (60-80%) was higher than that of fructose (30 to 40%) (Csamovsky, 1954). The overwintering adult solitary bees, Ceratina flavipes and C. japonica showed extraordinarily high

sugar levels (10-15% / body weight) whereas fructose comprised 45-77 mg / g body weight (Tamuo, 1964). Blum et al. (1962) found fructose, glucose and trehalose in the free form in all the main anatomical areas of the reproductive systems of the drone honey bee. These sugars were particularly greater in concentration in the drones leaving the hives than in the drones returning from flight. The same sugars were also found in the ejaculated semen of the drones.

The female moths of Agrotis segetum, Pyrausta nubilalis and Loxostege sticticalis consumed greatest quantity of sugars during the 4 to 5 post emergent days which were related with gonadal maturation (Mozhantshikov, 1930). Further, during the oviposition period the sugar consumption remained at a low level until death.

In tsetse fly, Glossina species the sugar level in the whole fly was found very low (Gaiy et al., 1959). However, glucose was slightly higher in concentration than trehalose. The level of glucose in the whole body of the blow fly Phormia regina was higher in the males than that of the females (Tate and Wimer, 1971). Further, the glucose concentration was lowest at the formation of the puparium (14 µg/insect), then it slightly increased after 24 hours (to 23 µg/insect); However, this sugar exhibited a sharp rise after the complete formation of the puparium (to 42 µg/insect). Further, it significantly dropped (to 27 µg/insect) just before the emergence of the

adults. But in Lucilia cuprina, although glucose concentration decreased during the larval-pupal transformation (about 20%) there was no further change up to emergence (Crosfton and Mirt, 1967).

In Periplaneta, the information of the reducing sugars is lacking. However, in the haemolymph and reproductive organs of Cimex species both glucose and trehalose were detected (Ano, 1972). However, trehalose was hydrolysed during the formation of active sperms and it was followed by oxidative utilization of the glucose.

FAT METABOLISM:

The study of fat content in insects was first made by Pellner (1834) in Bombyx mori, then by Dubois (1893) in Schistocerca gregaria. Since then several workers concentrated to investigate on various aspect of fat metabolism in a variety of insects. However, in the present account the information available in the last two decades is reviewed.

In the most primitive form of insects, Lepisma saccharina, approximately 8% of the body weight was made up of fat (Kinsella, 1969).

The adult desert locust, Schistocerca gregaria contained an average total fat content 9.6% of fresh body weight (Weis-Fogh, 1952). In the male, S. gregaria, during the first week

following emergence, there was rapid increase in the amount of body lipids which was followed by progressive depletion during the rest of the adult life (Odhiambo, 1966b and Walker et al., 1970). However, during the migrating swarm, the total fat content of the body in both sexes rose 4 to 6 fold than the original amount (Waloff, 1961).

During the adult life of Locusta migratoria migratorioides in both sexes, a rapid increase in total fat concentration occurred during the first two weeks following emergence (Chen, 1952). However, in the females a sharp reduction took place just before the oviposition of the first batch of the eggs which was correlated with the reduced feeding at this period whereas in the males the total fat content dropped with old age.

Thomson and Harvin (1953) found that in the american roaches, Periplaneta americana, the females had always slightly more lipids than the males but more pronounced difference occurred in the 3 week old adults and such sexual difference was also found in the nymphs.

The total fat content of the japanese beetle, Popillia japonica increased with age and reached a maximal level in the last larval stage which subsequently declined in the pupa and the adult (Ludwig and Rothstein, 1949 and Battista, 1954). Similar trend occurred in Tropodema armarium which showed significant differences in the lipid contents of the larval, the pupal and the adult stages (Rao and Agarwal, 1970).

Maximum lipid level was observed in the last larval instar (27% of fresh weight of the body), which decreased in the pupal stage and finally reached to a lowest level in the adults. Further, the diapaused larvae of T. granarium showed an enormous increase in the amount of fat as compared to the non-diapausing larvae (Kamawar and Nair, 1969). However, in the absence of food the fat concentration of the non-diapausing larvae depleted.

In Trogoderma lineatum, there was an increase in the total fat content of the males from 5 days after the start of attack whereas the females lost fat during the digging of the brood and oviposition in this equivalent period, then the level remained steady for 7 days and finally increased rapidly (Nijholt, 1969). Like Trogoderma granarium, during the development of the mealworm, Tenebrio molitor, the amount of lipid was highest in the 8th instar larvae, constantly remained at a lower level in the pupae and it was least in concentration in the adults, especially in the males (Horan, 1959 and Gourdoux, 1970). The lipid content also showed changes during the developmental stages of Rantus bimaculatus which maintained a constant level of lipids during the embryonic development, a fall during the hatching, progressive increase during the larval development and its utilization by the pupa (Arul Jin et al., 1971).

Nawanze et al. (1976) found that the normal and the flight forms of the adult cowpea weevils, Callosobruchus maculatus, within 24 hours after completing ecdysis showed

differences. The flight forms had nearly twice as much total body lipids as that of the normal forms. However, the females of both the forms contained more total lipids than that of the males.

The total lipid content of the queens of Bombus hypnorum and B. pratorum during the period of hibernation (Marillenu et al. 1974) had an increase whereas it significantly dropped during the post-hibernation period.

Among the Lepidoptera, changes in the lipid content was studied in detail in Hyalophora cecropia during the development by Gilbert and Schneiderman (1961) and Denrose and Gilbert (1964). It was recorded that the lipid content per individual larva increased with age and size. The newly hatched larva contained an appreciable amount of fat which increased as the larva grew older. Thus in the fully grown fifth instar larvaethe total lipid was five times than that of the early 5th instar larvae. At the time of pupation the lipid concentration was 5 to 7% of the fresh weight of the body, the male pupae having higher concentration than the female pupae, indicating a sexual dimorphism. After 2 days following emergence the males contained 30% more lipids than the females and this dimorphism continued during the adult life. However, in both sexes, the total lipids declined after 6th post emergent day.

Similar sexual dimorphism with regard to lipid concentration was also observed in the pupae and adults of Bombyx mori

(Niemienko et al., 1956) and Antheraea pernyi (Denyanovsky and Zubova, 1957). In B. mori, the newly emerged males had 48% lipids/gm dry weight while the females of the corresponding age contained only 25% lipids/gm dry weight of the body. Further, the females used 50% of their lipids against 30% by the males during the spinning period.

The sexual dimorphism in neutral lipids was also observed in the Indian meal moth, Plodia interpunctella (Yurkiewicz, 1969). During the last larval instar, the male larvae of P. interpunctella contained slightly more total neutral lipid/gm than that of the female larvae. In the pupa, the female catabolised more lipids than that of the males with the result the newly emerged females had much lower fat content as compared to that of the newly emerged males.

Wareheny and Sundaram (1971) during the metamorphosis of Dubleuma amabilis and Holocnera pulverea reported an increase in the percentage of the total fat at the transformation from the larva to the pupa and from the pupa to the adult, while a decrease occurred during the pupal period, suggesting that fat was thus synthesised in the newly formed pupa and adult, while it was utilised during the mid-pupal stage.

The total lipids of the female monarch butter fly, Danaus plexippus, during migration was studied by Genedella (1971). Further, in Plusia gamma, fat content of the body

increased during the larval life and decreased during the pupal stage (Wacnulay, 1974). Janda (1975) in Galleria mellonella correlated the growth of various organs in the initial period of the last larval instar to the increased lipid content in the body. In this species also during the spinning of the cocoon the fat content of the body decreased.

Changes in the lipid content of the body were also studied in some dipterous insects. Levenbook (1959) in Gastrophilus intestinalis larvae recorded highest concentration of fat in the matured larvae before metamorphosis. In Musca domestica also highest fat content was found in the last instar larvae before pupation but in the pupal stage it was significantly consumed (Ludwig et al., 1964). Further in Lucilia sericata lipid phosphorus content of the body reached a maximum level at larval-pupal transformation (De Costa and Birt, 1966). However, newly emerged adults had a low concentration which increased after 4 days.

Wakirtzi-Lemonias et al. (1969) observed higher lipid content in the older fruit fly, Dacus oleae than that of the younger flies of both the sexes.

In Culex pipiens fatigans also accumulation of lipids occurred during the larval development, which was utilized during pupal period (Kalra, et al., 1969). Similarly, in Drosophila melanogaster, total lipids proportionally increased with the enhancement of the body weight during the larval growth

(Church and Robertson, 1966). Further, in the adult Drosophila melanogaster, Fairbanks and Burch (1970) recorded initial fall in the lipid content but after 5 days following emergence the fat content of the females was consistently higher than that of the males.

Among the hemipterous bugs, in Dysdercus koenigii, the total neutral lipid content accumulated with age and size and maximum level reached in the last larval instar (Agarwal and Rao, 1969). Brown and Chippendale (1973) found synthesis and accumulation of lipids throughout the exceptionally long larval life of the periodical Cicada, Magicicada cassini. Further, the young female of this species had three times as much total lipids as that of the males.

II. MATERIALS AND METHODS

1. Breeding and stock culture:

Adult Dysdercus cingulatus Fabr. were initially collected from the cotton crop for mass rearing and maintenance of the stock culture. These were kept in the glass rearing jars measuring 8" x 4" in size. The bottom of the jars was filled with moist sand to a height of about two inches and top was covered by muslin cloth. These rearing jars were kept at 30°C ± 1°C and 70-80% relative humidity. Both the nymphs and the adults were fed on soaked cotton seeds every day. This stock culture provided the source of experimental insects.

2. Sampling of the nymphs and the adults:

From the stock culture, the freshly moulted nymphs to the 4th or the 5th instar as well as the newly emerged adults were sorted out daily between 9 A.M. to 12.00 noon. Such nymphs and the adults were regarded to be of zero age. Subsequently their age was determined at the time of experiment. The nymphs of similar age were kept in one jar, whereas each pair of the newly emerged adults was kept in a small rearing tube (in glass tube measuring 6" x 2" with 1" moist sand at bottom) and they were provided soaked cotton seeds daily. The maintenance of the age-wise adult pairs was also with a view to observe their mating and oviposition.

3A. Quantitative determination of the total proteins concentration:

a) Preparation of the homogenates.

Each nymph or the adult was weighed and singly homogenised in 1.0 ml of 1.0% NaCl solution. At a time homogenates of the whole body of two individuals of different age were prepared.

For estimating the total proteins of the ovarian tissue, two females of each age were dissected and after recording the anatomical condition of the ovaries, each pair of the ovaries was transferred to a weighed homogenising tube and dried at 35°C for 20 minutes to remove the traces of water. After weighing, the ovaries were homogenized in 1.0 ml of 1% NaCl solution.

All the homogenates were centrifuged at 4,000 r.p.m. for 15 minutes. The supernatants having the soluble proteins were used for the determination of the total proteins concentration and these were given a few drops of 5% mercuric chloride to prevent the enzyme activity on the proteins.

b) Chemical procedure:

The quantitative determination of the total proteins was based on the method of Lowry et al. (1952) with modification to suit its use on insect tissues.

Each supernatant was treated with 3.0 ml of 10% Trichloroacetic acid (AnalaR, B.D.H.), thoroughly shaken and centrifuged at 7,000 r.p.m. for 15 minutes to settle down the precipitate containing protein-fractions. This supernatant was rejected and the precipitate was washed with 0.1 N potassium acetate in absolute alcohol. Again it was centrifuged and the supernatant containing aminoacids was rejected. It was done thrice to remove all traces of aminoacids. The precipitate was further washed with alcohol and subsequently by ether to remove fats. Following this, each precipitate was dissolved in 1N NaOH by heating in the boiling water bath. This provided concentrated samples. From each concentrated sample, an aliquot (which varied in different cases) was drawn and mixed with distilled water to make the volume 1.0 ml. All such diluted aliquots were then mixed with 5.0 ml alkaline copper reagent (prepared by mixing 2% Na_2CO_3 in 0.1 N NaOH and 1% $CuSO_4$ in 2% potassium tartrate (50: 1 v/v ratio) and incubated at room temperature for 10 minutes. Following incubation 0.5 ml diluted (50%) 1 N Folin-ciocalteu reagent (Folin reagent) was added and mixed instantly to develop the colour. After 30 minutes colour density of each final solution was recorded from Systronic's photoelectric colorimeter against a blank sample by using a red filter. The blank sample consisted of 1.0 ml distilled water, 5.0 ml alkaline copper reagent and 0.5 ml diluted Folin reagent.

Table - 1

Serial dilutions, reagents and optical density of the standard curve for proteins

TREATMENT	SERIAL DILUTION									
	Blank	1	2	3	4	5	6	7	8	9
Quantity of standard protein solution (ml)	-	0.05	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8
Quantity of water added (ml) (to make equal volume i.e. one)	1	0.95	0.9	0.8	0.7	0.6	0.5	0.4	0.3	0.2
Reagent 'C' (ml)	5	wait for 10 minutes								
Folin Reagent (ml)	0.5	after 30 minutes								
Optical density	-	0.075	0.15	0.295	0.04	0.59	0.74	-	-	-
Protein concentration (mg)	-	0.02	0.04	0.08	0.12	0.16	0.20	-	-	-

Strength of stock protein solution : 10 mg/25 ml (Bovine serum albumin)

Fig. 1. Dilution curve for the determination of total proteins concentration per mg body or tissue weight in the nymphs and adults of *D. cingulatus*.

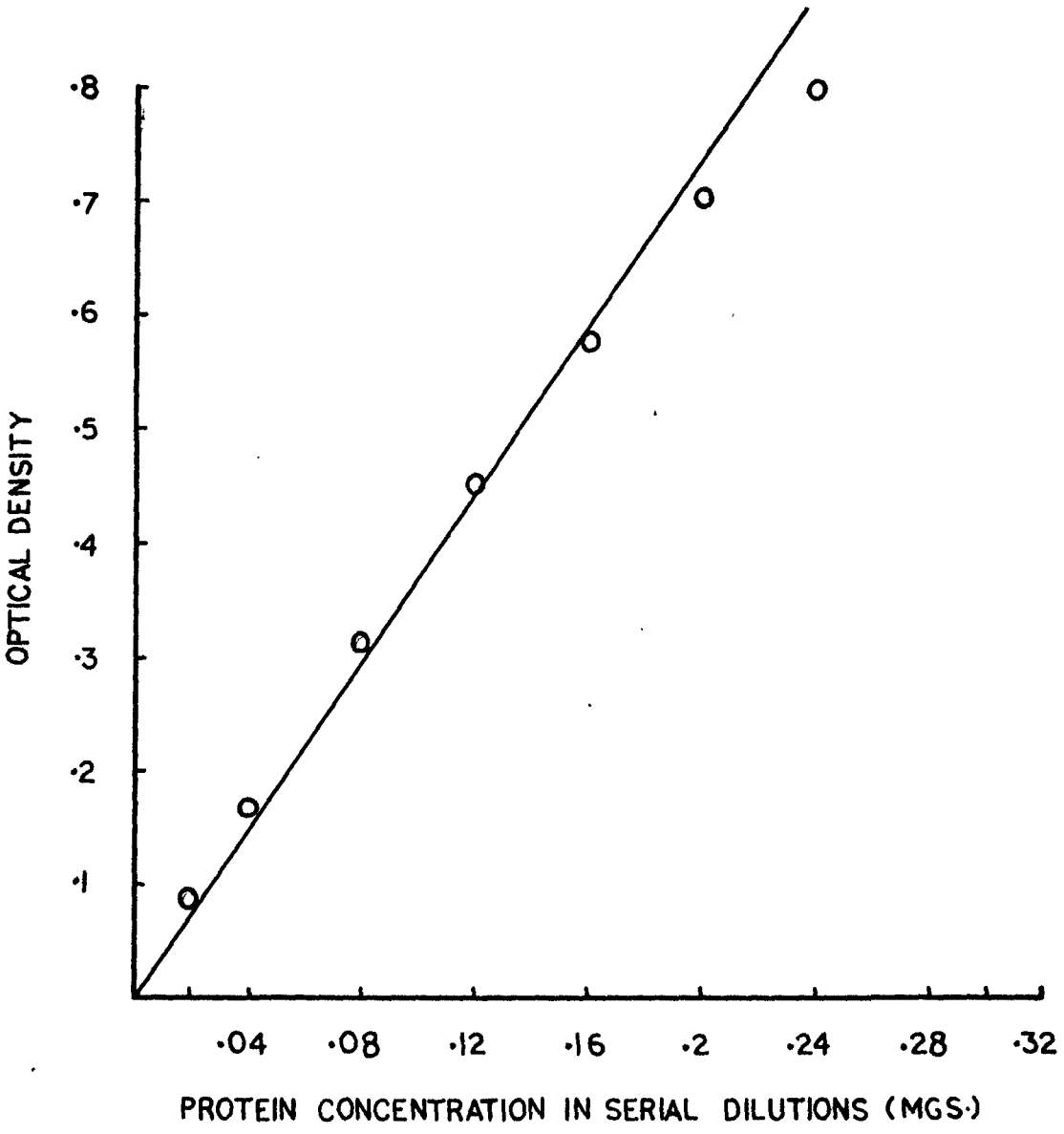

OPTICAL DENSITY
·8
·7
·6
·5
·4
·3
·2
·1
·04
·08
·12
·16
·2
·24
·28
·32
PROTEIN CONCENTRATION IN SERIAL DILUTIONS (MGS·)

FIG. 1

To calculate the quantity of the total proteins in different coloured solutions, a standard curve (Fig. 1) was plotted by using serial dilutions of bovine serum albumin of known strength (supplied by B.D.H., England). The optical density of the final coloured solution of the serial dilutions was plotted against the known quantity of the protein in the respective dilutions. The details of the serial dilutions and their optical densities are given in Table 1. Thus, the quantity of the proteins was recorded (x) in the aliquot taken (y). Further, the quantity of the total proteins in the aliquot per ml (z) was obtained (x/y = z). From this data the total protein concentration (TPC) was calculated in each precipitate which was dissolved in 5.0 ml NaOH (Z x 5.0 = TPC). Finally the protein concentration per mg of the body weight or per mg of ovarian weight was known by dividing the TPC with the body or the tissue weight of the adult or the ovary, respectively. The data were analysed statistically to appreciate the significant difference as described later.

B. Separation of different proteins of the ovaries:

Different proteins of the ovaries of the normal as well as that of the females treated with insecticides was made by polyacrylamide gel electrophoresis technique which was based on that of Davis (1964). The details are given below.

a) Dilution and Application of the insecticides:

In the present investigation, Lindane (γ -isomer of hexachlorohexane), a representative of the chlorinated hydro-

carbons (supplied by Sandoz (India) Ltd.) and Parathion (methyl parathion) a representative of organophosphorus compounds (supplied by Bayer (India) Ltd.) were selected for the study as these were the highly effective insecticides on D. cingulatus.

Both the insecticides were applied by the topical method on the 4 day old 5th instar nymphs of D. cingulatus. Technical Lindane (95%) was diluted in acetone (AnalaR) to 0.100, 0.050, 0.025, 0.0125 and 0.0062% concentrations. From each dilution, 1.0 µl was applied on the metathoracic pleuron of a nymph by a 26 gauge hypodermic needle of a glass syringe (1 ml Tuberculine) which was attached to a micrometer. For each dilution three replicates, each consisting of thirty nymphs were used and they were provided with soaked cotton seeds daily. The males and the females emerged from these treated nymphs were kept in separate jars. The LD_{50} of Lindane was 0.0125% based on the survival through the nymphal stage, complete emergence and longevity up to twelve days following emergence.

Technical Parathion (80%) was diluted to 0.125, 0.0625, 0.0312, 0.0156, 0.0078 and 0.0039% concentrations in acetone for comparable dilutions with that of Lindane, the LD_{50} value for Parathion was 0.0078%.

Thus on the basis of the LD_{50} values in all the future applications respective dilutions of Lindane and Parathion were

applied by the topical method as described above.

b) Preparation of the homogenates:

Following the emergence of the adults from the treated nymphs the ovaries of the females (affected females) were removed on the alternate days up to 12 days. Similarly the ovaries of the females from the normal nymphs of the corresponding age were also obtained. At a time, several females (both affected and normal) of each age were dissected and a constant weight of the ovarian tissue i.e. 100 mg (pooled) was used for the separation of the proteins to minimise the experimental error. The pooled ovarian tissue either from the affected or the normal females were crushed and homogenised in 1.0 ml of 1.0% NaCl in buffer solution pH 8.3 (used for electrophoresis). The homogenates were centrifuged at 7,000 r.p.m. for 15 minutes and the supernatants carrying the soluble proteins were used for the electrophoretic separation of different proteins.

c) Procedure for the separation:

As mentioned earlier, the separation of the proteins was made in the acrylamide gel, therefore, the gel tubes (glass tubes measuring 7 cm long; with 0.6 mm inner diameter) were first washed with surf washing powder and then with potassium dichromate solution in sulphuric acid and finally rinsed with deionised water. Further, these tubes were siliconised with 1% solution of silicol (Clay Adam, N.J.). The polyacrylamide gel column in the tube consisted of 3 sections: first, at the top

a large pore solution containing the protein sample, second in the middle a large pore spacer gel, and finally at the bottom a small pore gel in which electrophoretic separation took place.

Acrylamide, N,N'Methylenebisacrylamide (Bis), Riboflavin and N,N,N',N'-Tetramethyl ethylene diamine (Temed) were obtained from Koch-Light Laboratories, England whereas Ammonium persulphate and Glycine from Riedel, Germany. Tris (hydroxymethyl) aminomethane was supplied by the BDH (England).

The following reagents were employed and prepared as follows:

Solution 'A' (pH 8.9)

1N HCl	48.00 ml
Tris	36.69 gm
Temed	0.23 ml
Distilled water to	100.00 ml

Solution 'B' (pH 6.7)

1N HCl	48.00 ml
Tris	5.98 gm
Temed	0.46 ml
Distilled water to	100.00 ml

Solution 'C'

Acrylamide	28.00 gm
Bis	0.735gm
Distilled water to	100.00 ml

Solution 'D'

Acrylamide	10.00 gm
Bis	2.50 gm
Distilled water to	100.00 ml

Solution 'E'

Riboflavin	4.00 mg
Distilled water to	100.00 ml

Solution 'F'

Sucrose	40.00 gm
Distilled water	100.00 ml

Small pore gel solution: (pH 8.8-9.0)

1 part solution A

2 parts solution C

1 part distilled water

This was mixed with an equal quantity of 0.14% of ammonium persulphate solution.

Large pore gel solution (pH 6.6-6.8)

1 part solution B

2 parts solution D

1 part solution E

4 parts solution F

Stock Buffer Solution for Reservoirs: (pH 8.3)

Tris	6.0 gm
Glycine	28.8 gm
Distilled water to	1.0 litre

(1/10th strength of this buffer solution was diluted with distilled water and used. Further, pH of the above solution was adjusted by titrating with N HCl).

Each gel tube was first filled with 0.85 ml small pore gel solution. This solution was clearly overlaid with 0.1 ml distilled water and allowed to stand for 30 minutes. Then water layer was removed and 0.15 ml large pore gel solution was added and carefully overlaid with 0.1 ml of distilled water. A day light fluorescent tube lamp was placed over the tubes for 15 minutes for the polymerization of the large pore gel. The water layer then was removed and 40 µl sample of proteins homogenate mixed with 0.15 ml large pore gel solution was added on the top. The sample layer was again polymerised for 15 minutes in the manner described above. The remaining space in each gel tube was finally filled with buffer solution (pH 8.3).

An alternative and relatively simple method of sample application was also used, in which the use of sample with spacer gel (large pore gel solution) was omitted. Instead the sample which consisted of 40.0 µl proteins homogenate was mixed with 3.0 µl bromophenol blue (0.05%) and one drop of glycerol was applied to each gel under a layer of buffer just above the small

Fig. 2. Disk electrophoresis apparatus.

UR - upper reservoir

LR - lower reservoir

G - gradients

C - cathode

A - anode

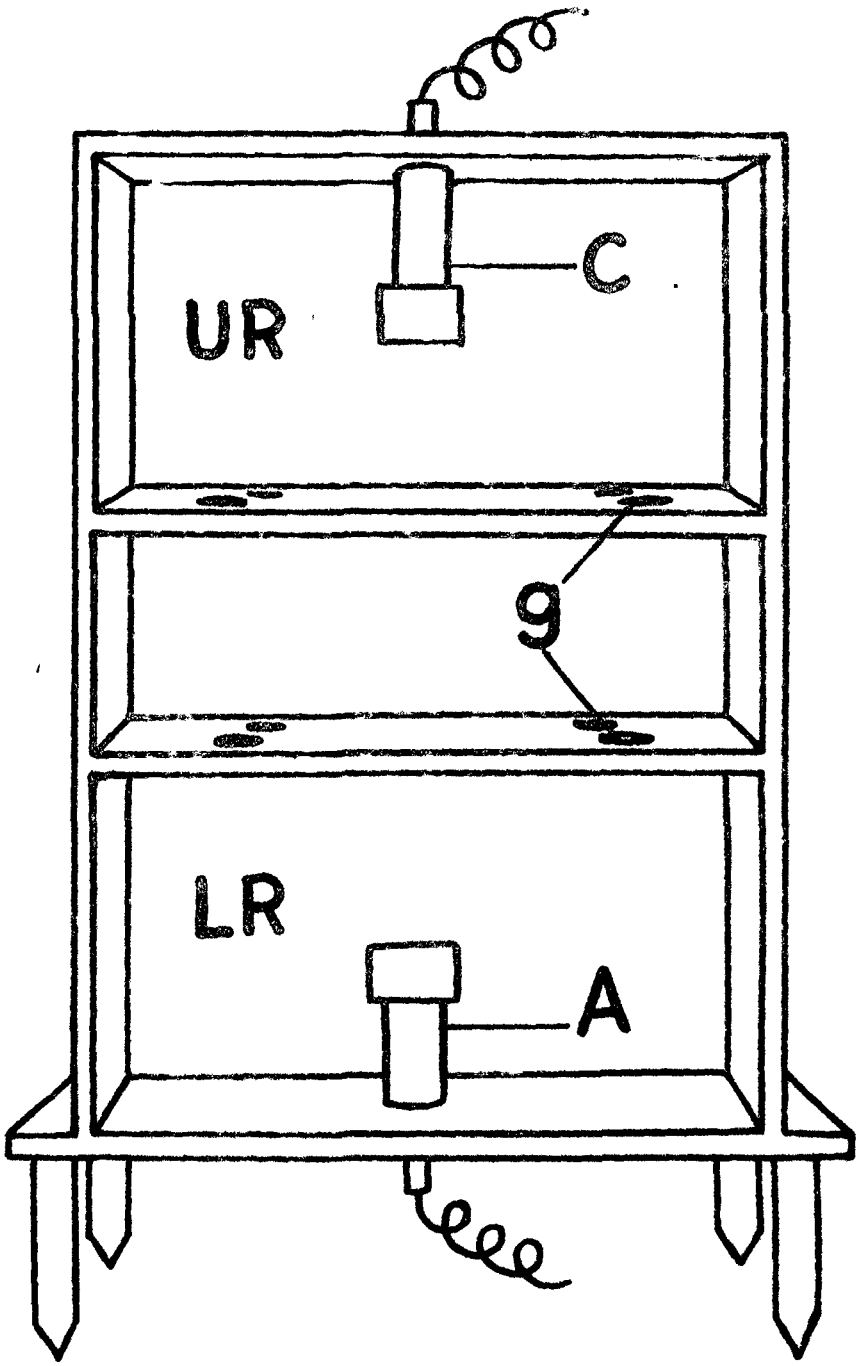


Fig. 2

pore gel solution. This method of sample application gave better resolution and more over the duration of each electrophoretic run was reduced to half.

The disk electrophoretic apparatus (Fig. 2) is made of perpex and consists of two chambers arranged vertically an upper and a lower reservoir which are connected with each other through separate coverings in the centre having grommets for insertion of the gel tubes. The upper reservoir is connected with cathode whereas lower one with that of anode.

The samples were separated electrophoretically at room temperature (25-27°C). The tubes were vertically inserted into the grommets of the upper buffer reservoir of the electrophoretic chamber. This reservoir was filled with 200 ml stock buffer solution(9pH 8.3). The lower reservoir was also filled with the same buffer so that 1/2 inch of the lower end of the tubes were dipped into the buffer. The power supply was connected, cathode to the upper reservoir and a constant current of 3 m.A/tube was supplied. The protein samples were allowed to run for one and a half hour when the tracking bromophenol blue dye band reached near the bottom (about 5 mm from the lower gel end) of the tube. Then gel tubes were removed from the grommets and gel columns were removed from the glass tubes by gently rimming them with 22 gauge needle through which a thin stream of water was made to pass. After the removal of the gels, a metalic marker was inserted into the tracking dye band because it disappeared

Fig. 3. A gel column showing different sections.

A - Section with large pore gel solution.

B - Section with small pore gel solution.

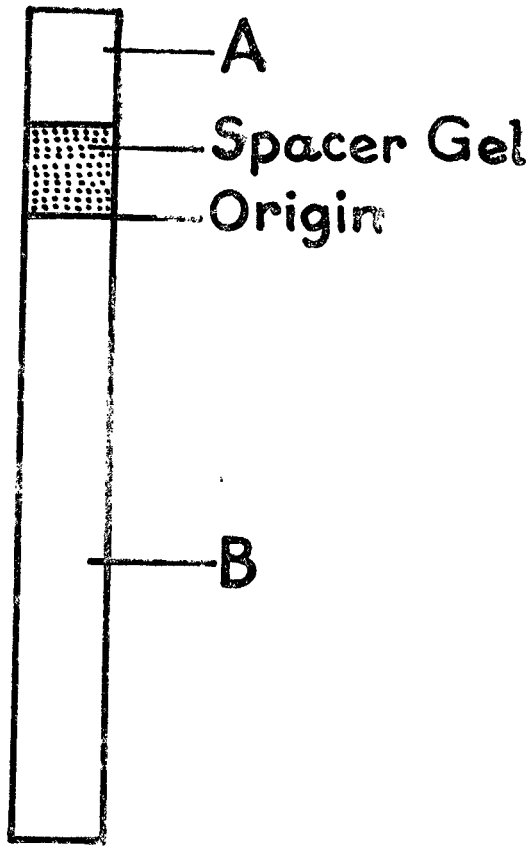


Fig. 3

during the fixation process. The removed gels were kept in 12.5% TCA solution for 45 minutes to fix the protein bands on the gel.

Further, the protein bands were stained in 0.01% coomassie brilliant blue dye (1%) in 12.5% TCA by keeping the gels into the dye for an hour. Following this the gels were destained, first by keeping in 10% TCA for 24 hours and then in 7% acetic acid which also served as preservative for the entire gel. The gels having the coloured protein bands were photographed against diffused light. Since certain minor bands could not be clearly photographed owing to faint colour, therefore, tracing and retouching from the photographs was also essential to represent all the bands through diagrams.

The relative electrophoretic flow (Rf) value of each separated protein band was calculated as follows:

$$Rf = \frac{\text{Distance travelled by protein band from origin}}{\text{Distance travelled by tracking dye from origin}} \times 100$$

The junction of the spacer and separation gels was considered as the origin (Fig. 3).

4. Estimation of total reducing sugars:

a) Preparation of homogenates:

The total reducing sugars (TRS) was determined per mg of the whole body of the individuals of the 4th and the 5th instar nymphs as well as in the adult males and females of

different age. Therefore, each nymph or the adult of different age was first weighed and then separately homogenised in 1.0 ml NaCl (1.0%) solution. At a time, two members of each stage and age were homogenized.

Similarly for the determination of the total reducing sugars (TRS) per mg of the ovarian weight of the individual female from emergence to the 12 day old age, at a time, the paired ovaries of two females of each age were dissected and transferred to a filter paper for one minute to remove the adhering water. Then each pair was weighed and homogenised in 1.0 ml NaCl (1.0%) solution.

All the homogenates were centrifuged at 4,000 r.p.m. for 15 minutes and the supernatants thus obtained were used for TRS estimation.

b) Chemical procedure:

The quantitative determination of the total reducing sugars(TRS) in the tissue homogenates was based on the method of Sumner (1925). It was found to be quite sensitive to detect the traces of the reducing sugars in the homogenates. However, the quantity of the sample, reagent and other procedure was modified to suit to the present investigation.

From each supernatant an aliquot of 1.0 ml was mixed with 3 ml dinitrosalicylic acid reagent (Sumner reagent). The mixture was heated for 25 minutes in the boiling water bath to

Table - 2

Serial dilutions, reagents and optical density of the standard curve for glucose

TREATMENTS	SERIAL DILUTIONS									
	Blank	1	2	3	4	5	6	7	8	9	10
Quantity of standard glucose solution (ml)	-	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	0.10
Quantity of water added (ml) to make the equal volume i.e. 1 ml	1	0.9	0.8	0.7	0.6	0.5	0.4	0.3	0.2	0.1	-
Sumner Reagent (ml)	3	Heated for 25 minutes									
		Cooled in running water for 15 minutes									
Quantity of distilled water added (ml)	6										
Optical density	-	0.035	0.13	0.27	0.36	0.475	0.5	0.59	0.69	-	-
Glucose concentration (mg)	-	0.03	0.06	0.09	0.12	0.15	0.18	0.21	0.24	-	-

Strength of the glucose solution : 30 mg/100 ml

Fig. 4. Dilution curve for the determination of total reducing sugars per mg body or tissue weight in the nymphs and adults of *D. cingulatus*.

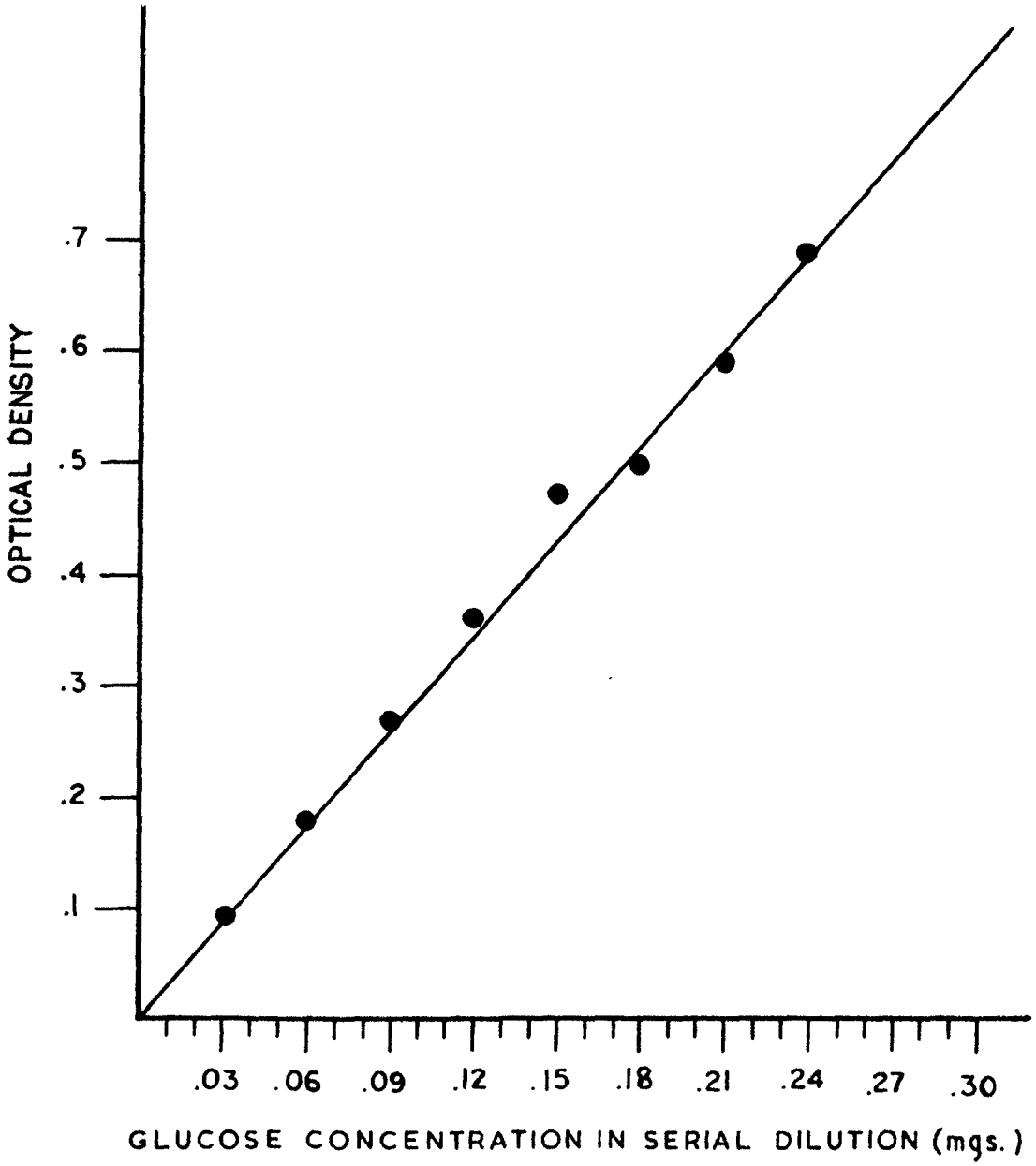

OPTICAL DENSITY
.7
.6
.5
.4
.3
.2
.1
.03
.06
.09
.12
.15
.18
.21
.24
.27
.30
GLUCOSE CONCENTRATION IN SERIAL DILUTION (mgs.)

FIG. 4

develop the colour, then cooled in the running cold water for 15 minutes. Finally, 6 ml distilled water was added and thoroughly mixed in each coloured final solution to make up the volume 10 ml. Simultaneously, a reagent blank was also prepared which contained 1.0 ml distilled water instead of homogenate sample. Further procedure was similar to that for other samples. The colour density of the final solutions was known against the reagent blank solution on a spectrophotometer (Spectronic-20, Bosch and Lomb) using a green filter.

The quantity of the reducing sugars in each sample was calculated by means of a standard curve (Fig. 4) which was plotted by obtaining colour density of the serial dilutions of Glucose (AnalaR) of known strength. The development of the colour in the serial dilutions of the glucose was done in the same manner and by the same procedure as described before. However, the volume of each serial dilutions was made to 1.0 ml by adding the required quantity of distilled water (Table 2). Finally the total reducing sugars concentration per mg of the body weight or the ovarian weight was calculated.

5. Estimation of the total fat concentration:

The estimation of the total fat concentration (TFC) in the whole body of the 4th and 5th instar nymphs as well as adults of both sexes of *D. cingulatus* was based on the soxhlet extraction method (Method in Enzymology Vol. III).

In the nymphs this observation was made with respect to each age from the time of moulting to the 4th instar to

metamorphosis. Whereas in the adults, the TFC was determined in the whole body of the adults belonging to alternate age from emergence to the 12 days which consisted of two sexual cycles in this species. With respect to each stage individuals of an age were together weighed to one gramme and then dried at 70°C for 40 hours. The dried tissue was kept in a desiccators for 24 hours to cool, then thoroughly ground to fine powder in a dry mortar with the help of the weighed quantity of anhydrous sodium sulphate to make the grinding easy. Then transferred to a thimble which was then placed in a Soxhlet extraction tube filled with Petroleum ether (B.P.H.) and connected with a measured flask to collect the extracted fat.

The extraction of the fat was allowed on continuous heating for 10 hours on a boiling water bath. Refilling of the petroleum ether was continuously done during the extraction period. All traces of ether were finally removed by keeping the extracted fat at 95°C for 24 hours. Further, the dried fat extracts were kept in the desiccators for 2 to 4 hours to stabilise the weight. Then the weight of the extracted total fat was known in the known weight of the body tissue.

6. <u>Statistical interpretation of data:</u>

The detailed data were given in the Appendix tables whereas the consolidated results were reffered in the text. The mean values of a series of arithmetical figures were known and standard error for each mean value was also calculated.

Following statistical formula was used to calculate the standard error (S.E.) of the arithmetical means.

$$S.D. = \sqrt{\frac{T}{n-1}} \quad \left(T = \Sigma x^2 - n\bar{x}^2\right)$$

$$S.E. = \frac{S.D.}{\sqrt{n}}$$

Where Σx^2 is the sum of the squares of the individual arithmetical figure in each experiment, n denotes the total number of the samples and $\bar{x}^2$ is the square of the arithmetical mean.

It was desirable to test the mean values statistically to appreciate the significant differences between the mean values for various days or between any two mean values. For this purpose, the zero age, was reckoned as " control " and the statistical technique of variation significance ('t' test) was applied as follows according to P.G. Hoel (1962).

$$t^2 = \frac{(\bar{x}_1 - \bar{x}_2)^2}{s^2\left(\frac{1}{n_1} + \frac{1}{n_2}\right)}$$

$$s^2 = \frac{T + T'}{n_1 + n_2 - 2}$$

$$\left[\begin{array}{l} T = \Sigma x^2 - n_1 \bar{x}_1^2 \\ T' = \Sigma x'^2 - n_2 \bar{x}_2^2 \end{array}\right]$$

$$t = \sqrt{t^2}$$

where n_1 = number of observations in first sample, $\bar{x}_1$ = mean of

first sample and T = sum of squares of deviations from the mean (first sample).

The tabulated value of " t " differs in each experiment according to different degrees of freedom (number of samples) and percent level of significance. If the calculated value of " t " exceeded than that of the tabulated 't' value, ascribed further in each table of the text accordingly, it was inferred that the difference between the mean values was statistically significant otherwise it was an insignificant difference. The significant values were marked with a star (*) in each table.

7. Illustrations:

Although consolidated tables of data have been referred in the text of the present thesis to appreciate the variations in different experimental conditions, some important correlations between different observations have been graphically illustrated and by appropriate histograms.

The anatomical details of the female reproductive organs and the ovarioles have been given with the help of a Camera Lucida.

III. CHANGES IN THE WHOLE BODY WEIGHT OF THE 4th AND THE 5th INSTAR NYMPHS AND THE ADULT D. CINGULATUS RELATED WITH AGE AND METAMORPHOSIS.

The whole body weight of the growing nymphs changes and thus to appreciate the variations in the metabolites corresponding changes in the body weight were important to record. Therefore, the body weight of the nymphs which moulted to the 4th-instar and thereafter up to the metamorphosis was daily recorded in 20 individual larvae of each age. Similarly, after the metamorphosis weight of atleast 20 members of each sex of different age covering a period of 12 days was recorded (Appendix table 1 to 2).

The duration of the 4th-instar was 6 days and during this period it was observed that the whole body weight of different nymphs of the same age varied. The mean value of the whole body weight in this instar significantly increased from zero to six days (Table 3).

In the newly moulted 4th instar nymphs (zero age), the whole body weight ranges between 5.5 mg to 14 mg per nymph (mean 8.875 ± 0.542 mg per nymph). Further, the mean body weight increases with that of age and size of the body. Within the 24 hours following moulting, some growth takes place and the weight ranges between 5.0 mg to 14.5 mg per nymph (average, 10.10 ± 0.643 mg per nymph). The two day old nymphs have more

Table - 3

Changes in the total body weight of the 4th instar nymph of *D. cingulatus* related with age and moulting

Age (days)	Mean value of total body weight (mg)	S.D.	't' value	
Zero	9.875	± 0.542	Control	
1	10.100	± 0.643	1.655	15.193*
2	21.575	± 0.336	17.092*	4.724*
3	25.010	± 0.566	21.722*	1.301
4	26.720	± 0.352	24.024*	0.403
5	27.025	± 0.408	24.427*	1.806
6	29.110	± 0.714	27.240*	
Moulted 5th instar	23.495	± 0.672	5.726*	

(Table value of 't' at 5% level 30 = 2.021)

than double weight as compared to that of one day old individuals and it ranges between 18.5 mg to 25 mg per nymph (mean, 21.575 ± 0.396 mg per nymph). During the next 24 hours the rate of the weight increase becomes slow although it is significant (Table 3). However, the nymphs were much heavier than those of the preceding age because the range of their weight was between 20.0 mg to 30.0 mg (mean, 25.010 ± 0.566 mg). During the next three days the rate of increase in weight was smaller than before (Table 3). However, the six day old nymphs show variation of weight between 22.0 mg to 37.0 mg (mean, 29.110 ± 0.714 mg). Thus maximum body weight was attained on the 6th post-moult day i.e. just before the moulting to the 5th or the final instar (Fig. 5). Further, the mean range of variation in the whole body weight among the 4th instar nymphs from the zero to the 6 day old age was between 8.875 ± 0.542 mg to 29.11 ± 0.714 mg per nymph and the maximum increase in weight was more than three times.

The arithmetical mean values indicate insignificant increase of the weight in one day old nymphs. However, in the nymphs of the subsequent age there is significant increase in the body weight as compared with that of zero age nymphs but the rise is initially rapid and then gradual in the later part of the instar (Fig. 5).

During the process of moulting from the 4th instar to the 5th instar, there was a remarkable fall in the whole body weight and the newly moulted 5th instar nymphs were lighter than

Table - 4

Changes in the total body weight of the 5th instar nymph of *D. cinculatus* related with age and metamorphosis

Age (days)	Mean value of total body weight (mg) S.E.		't' value
Zero	23.495	± 0.672	Control
1	26.075	± 1.021	1.515
2	39.605	± 1.014	7.626*
3	50.255	± 1.159	9.535*
4	66.465	± 1.118	6.139*
5	68.025	± 1.091	15.722*
6	73.485	± 0.941	9.524*
7	77.125	± 1.518	25.246*
8	85.680	± 1.879	1.306
Emerged female	64.041	± 1.477	26.633*
Emerged male	55.577	± 0.930	2.737*
			29.371*
			2.138*
			31.509*
			5.029*
			36.539*
			9.176*
			24.573*

(Table value of 't' at 5% level 38 = 2.021)

the fully grown nymphs of the 4th instar (Fig. 5). This difference was statistically significant (Table 3).

The duration of the 5th instar was 8 days after which the nymphs metamorphosed to adults. In these nymphs within 24 hours following the moult there was little change in the weight which was statistically insignificant. Later, there was generally steady progress in growth. However, after 4 days, the rate of this progressive increase in weight became lower (Fig. 5).

In the newly moulted 5th instar nymphs, the body weight varied from 10.0 mg to 29.0 mg per nymph (mean, 23.495 ± 0.672 mg). One day old nymphs weighed from 14.5 mg to 30.5 mg with an average 26.075 ± 1.021 mg but the increase in the beginning was slow and statistically insignificant (Table 4). Like the 4th instar nymphs, in those of the 5th instar also the body weight rapidly increased after 24 hours following moulting and such trend continued up to 4 days when the mean body weight was 66.465 ± 1.118 mg which was more than double of the minimum weight during this instar. The rate of increase in 2, 3 and 4 day old nymphs was respectively higher significantly than that of the younger nymphs (Table 4). Afterwards, although the weight was continuously built up, the rate of increase became slower in the second half of this instar. As compared with the mean body weight of the zero age nymphs those of the later half of the instar was significantly higher but although the

Fig. 5. Changes in the total body weight of the 4th and the 5th instar nymphs and adults of *D. cingulatus*.

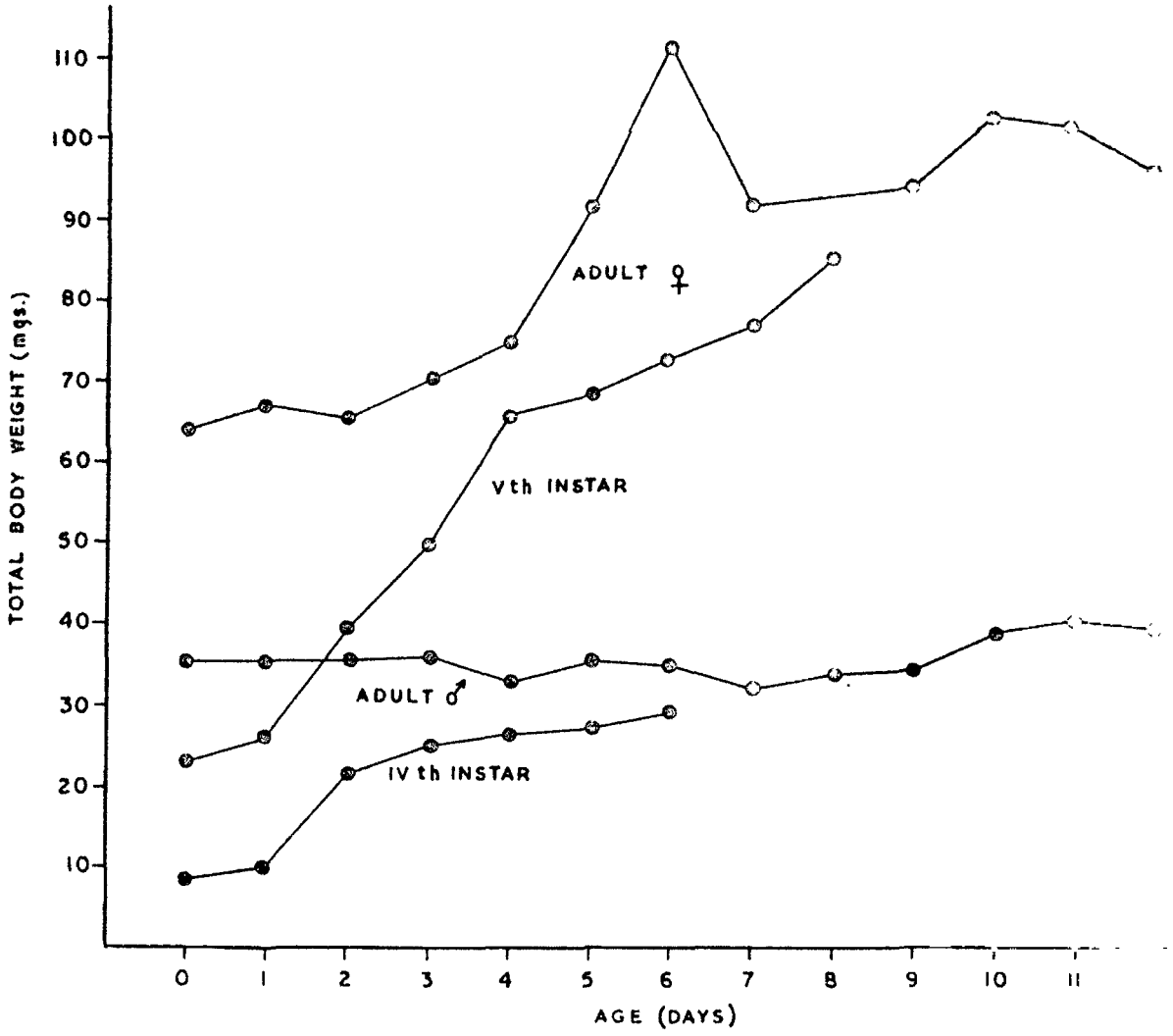

TOTAL BODY WEIGHT (mgs.)
110
100
90
80
70
60
50
40
30
20
10
ADULT ♀
Vth INSTAR
ADULT ♂
IV th INSTAR
0
1
2
3
4
5
6
7
8
9
10
11
AGE (DAYS)

FIG. 5

values were lower when compared with the mean values of the subsequent two age groups (Table 4). However, maximum body weight was recorded on the 8th post-moult day (85.600 ± 1.879 mg) which was more than three times the weight of the zero age nymphs in this instar (Table 4). Therefore, the range of variation in the whole body weight among the nymphs of the 5th instar was from 23.495 ± 0.672 mg to 85.600 ± 1.879 mg. Further, at the end of the nymphal life the nymphs gained about ten times weight than that of the newly moulted 4th instar nymphs (Table 3 and 4).

It was observed that increase in the whole body weight from first day to the middle of the instar was 42.97 mg and later it was 19.215 mg, providing an evidence of faster growth and metabolism in the first half of the instar. In the newly emerged adults, total body weight was significantly lower than that of the fully grown nymphs before metamorphosis. This decline was more remarkable in the males than in the females (Fig. 5).

Although the whole body weight of the individual adult D. cingulatus (both sexes) varied within the same age group, it was found that the mean value with respect to males did not appreciably increase in older males as far as 9 days following emergence. However, in the males of subsequent age groups there was more increase in the average weight of the whole body but it was statistically insignificant (Table 5).

Table - 5

Changes in the total body weight of adult *D. cingulatus* in relation to age and reproduction

Age (days)	MALE: Mean value of total body weight (mg) S.E.		't' value	FEMALE: Mean value of total body weight (mg) S.E.		't' value
Zero	35.577	± 0.930	Control	64.041	± 1.477	Control
1	35.650	± 1.148	0.049 / 0.080	67.412	± 1.119	0.675 / 0.324
2	35.522	± 1.240	0.030 / 0.297	65.795	± 1.501	0.351 / 0.971
3	36.004	± 0.964	0.264 / 1.567	70.645	± 2.303	1.322 / 0.957
4	33.472	± 1.362	1.304 / 1.304	75.425	± 3.336	2.279* / 3.242*
5	35.722	± 1.091	0.089 / 0.161	91.616	± 3.011	5.522* / 4.055*
6	35.463	± 1.200	0.070 / 1.937	111.866	± 3.280	9.578* / 3.951*
7	32.018	± 1.065	1.505 / 1.474	92.133	± 3.629	5.626* / 1.554
8	34.395	± 1.101	0.732 / 0.210	84.375	± 4.693	4.072* / 2.078*
9	34.731	± 1.192	0.846 / 2.657*	94.750	± 4.148	6.150* / 1.688
10	39.022	± 1.171	1.134 / 1.127	103.183	± 3.992	7.039* / 0.162
11	40.840	± 1.226	1.260 / 0.613	102.370	± 3.657	7.676* / 2.093*
12	39.850	± 1.089	1.647	96.912	± 4.704	6.583*

(Table value of 't' at 5% level 42 (males) and 46 (females) = 2.021

In the newly emerged males, the whole body weight ranged between 30.0 mg to 45.0 mg whereas that of the females of the corresponding age it was between 49.0 mg to 75.2 mg having the mean values 35.577 ± 0.930 mg and 64.041 ± 7.477 mg respectively. Thus, the average weight of the newly emerged females was nearly double of that of the males (Fig. 5). Later, in both the sexes the body weight increases. It was found that in the females of an age group the variations of weight was more appreciable (Appendix table 2) than that between the males of the same age. The range of variation in the weight of the males was between 32.010 ± 1.069 mg to 40.040 ± 1.226 mg and there were insignificant changes up to 12 days indicating almost a constant maintenance. But the males after 9 days put up some weight which is an insignificant increase (Fig. 5 and Table 5). In the females, within the first two days following emergence, there was insignificant increase in the weight. But later, significant rise continued and the maximal weight was gained by the 6 day old females (111.066 ± 3.280 mg) (Fig. 5). Afterwards, this weight significantly declined and remained low for a couple of days (Table 5). Then increased again and another level of maximum weight (103.103 ± 3.992 mg) was recorded in 10 day old females. It was again followed by a fall on the 12th post emergent day. The range of variation in the whole body weight of the females from zero to 12 day old life was between 64.041 ± 1.477 mg to 111.066 ± 3.280 mg. It was interesting to observe that in case of the females, first peak

of maximal weight was significantly higher than that of the second peak (Table 5). Further, the newly emerged males were lighter than the females of the corresponding age. The maximum weight of a male never compared with the lightest female (Table 5). This difference may be taken as dimorphic character.

IV. CHANGES IN THE WEIGHT OF THE OVARIES OF *D. CINGULATUS* RELATED WITH AGE AND REPRODUCTION.

As described earlier the mean values of the whole body weight of the males did not appreciably increase related with age. However, that of the females showed significant changes which could be ascribed to the growth and development of the ovaries. Therefore, it was necessary to observe the changes in the ovaries of the females belonging to different age from the time of their emergence.

The weight of each pair of ovaries of individual female of the same age varied (Appendix table 3). In the newly emerged females the mean of the weight of a pair of fresh ovaries was 12.81 ± 1.235 mg which was about one fifth of the total body weight whereas the dry weight of that pair of ovaries was 7.2 ± 1.412 mg i.e. about one ninth of the total body weight. Thereafter, the ovaries further gained weight and there was significant rise in the weight of fresh and dried ovaries from the females of three to six day old and maximum weight was attained by the ovaries of 6 day old females (55.64 ± 2.507 mg and 49.5 ± 4.053 mg respectively). Thus, at the time of first peak of body weight, the weight of the ovaries was about half of the body weight (Table 5 and 6, Fig. 6). After oviposition of the first batch of eggs, on the 7th or 8th post-emergent day, the weight of the ovaries significantly dropped and about two third of the weight was occupied by the eggs (Table 6).

Table - 6

Changes in the weight of ovaries of *P. cingulatus* related with age and reproduction

Age (days)	Weight of dried ovaries (Mean values mg) S.E.	't' value	Weight of fresh ovaries (Mean values mg) S.E.	't' value
Zero	7.2 ± 1.412	Control	12.81 ± 1.235	Control
1	9.1 ± 1.394	0.421	11.19 ± 0.716	0.439
2	8.5 ± 1.360	0.311	15.61 ± 1.282	1.015
3	22.7 ± 1.686	6.620*	28.62 ± 1.177	7.471*
4	27.1 ± 3.796	1.060	35.24 ± 2.303	1.859
5	34.9 ± 3.110	1.589	33.37 ± 2.690	0.883
6	49.5 ± 4.053	2.857*	55.64 ± 2.507	4.696*
7	11.7 ± 1.291	0.087*	20.91 ± 1.646	11.585*
8	20.6 ± 5.638	2.897*	33.03 ± 2.693	3.839*
9	20.3 ± 9.648	0.026	36.29 ± 2.686	0.856
10	31.9 ± 2.473	0.351	44.26 ± 3.032	1.702
11	37.7 ± 3.935	1.247	33.39 ± 5.402	1.641
12	13.4 ± 3.774	4.456*	22.63 ± 1.508	5.191*
		1.375		2.749*

(Table value of 't' at 5% level 10 = 2.101).

Fig. 6. Changes in the weight of the ovaries (fresh and dry) of the female *D. ginguletus*.

Fresh weight ·-o-·

Dry weight -●-

Body weight of the female -▲-

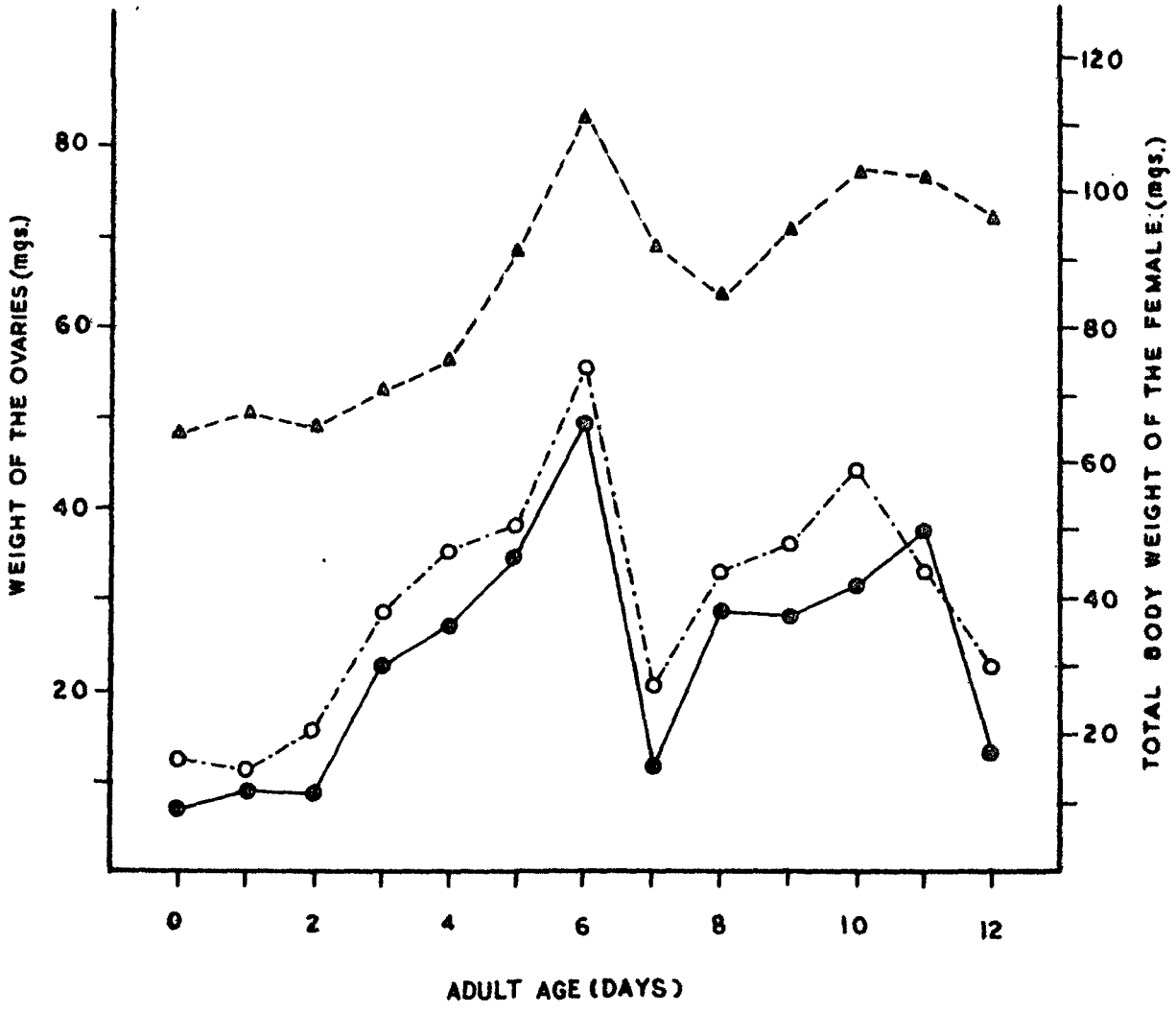

WEIGHT OF THE OVARIES (mgs.)
TOTAL BODY WEIGHT OF THE FEMALE (mgs.)
ADULT AGE (DAYS)
0
2
4
6
8
10
12
20
40
60
80
20
40
60
80
100
120

FIG. 6

Another significant rise took place from the 8th post-emergent day (Table 6). The second peak of maximum weight in the fresh (44.26 ± 3.832 mg) and dried ovaries (37.7 ± 3.939 mg) reached in 10 and 11 day old females (Fig. 6). Thereafter, the ovarian weight further declined as the female oviposited second batch of eggs. Like the changes in the body weight of the females, those of the ovaries were also significant after 2 days following emergence and likewise, the first peak of maximum weight was higher than that of the second peak (Fig. 6). The difference between the first and the second peak suggested that the second phase of growth and maturation involved less number of eggs than in the first oviposition. It was also interesting to record that both the peaks of ovarian weight were higher than the weight of the males of the respective age (Fig. 5 and 6).

From the above observations, it is clear that in the present controlled conditions of temperature and humidity, the two peaks of the body weight of the females correspond to fully matured condition of the ovaries. Whereas, subsequent fall in the body weight was related to the period of oviposition of the eggs. Thus, the changes in the weight of the ovaries were almost parallel to those of the whole body of the females (Fig. 6).

The ovarian weight after the oviposition of the second batch of eggs (22.88 ± 1.508 mg) was still significantly higher than the initial weight (12.81 ± 1.235 mg) in the newly emerged

females. This confirms an overall growth in the ovarian tissue following emergence and also the fact that in this bug commencement of growth and maturation of the eggs is largely imaginal process.

V. CHANGES IN THE CONCENTRATION OF PROTEINS IN THE WHOLE BODY OF THE 4th AND 5TH INSTAR NYMPH OF *D. CINGULATUS* RELATED WITH AGE, MOULTING AND METAMORPHOSIS.

The concentration of the total proteins (TPC) was determined in the whole body as per mg body weight in the 4th and the 5th instar nymphs of *Dysdercus cingulatus* of different age. The individual nymphs of the same age showed variation in the TPC. Thus mean values of TPC of ten nymphs of each age was obtained to compare the data.

In the newly moulted (zero day) 4th instar nymphs, TPC varied between 0.0113 mg/mg and 0.0205 mg/mg among the ten nymphs (Appendix table 4) and the mean value was 0.016 ± 0.0028 mg/mg body weight. Within the next 24 hours protein concentration did not appreciably increase because it ranged from 0.0117 mg/mg to 0.0285 mg/mg and the mean value was 0.017 ± 0.0019 mg/mg body weight. This change was also statistically insignificant. However, among the 2 day old 4th instar nymphs significant increase in TPC took place and it varied between 0.0129 mg/mg to 0.0282 mg/mg with a mean value of 0.023 ± 0.0020 mg/mg body weight. Addition of proteins in the body tissue of these nymphs continued and significant rise was recorded in the 3 day old nymphs which had a minimum of 0.0292 mg/mg and maximum of 0.0382 mg/mg (Appendix table 4) and the mean value was 0.033 ± 0.0011 mg/mg body weight. This

Table - 7

Changes in the total protein concentration in the body of the 4th instar nymph of *D. cingulatus* related with age and moulting.

Age (days)	Mean weight of protein/mg of body weight (mg/mg) S.E.			't' value	
Zero	0.016	±	0.0023	Control	
1	0.017	±	0.0019	0.350	0.012
2	0.023	±	0.0020	2.500*	0.035
3	0.033	±	0.0011	6.093*	0.0032
4	0.036	±	0.0023	7.168*	0.00035
5	0.035	±	0.0014	6.010*	0.005
6	0.039	±	0.0019	0.343	
Moulted 5th instar	0.023	±	0.0013	5.914*	

(Table value of 't' at 5% level 18 = 2.101).

is almost double of the TPC of the newly moulted 4th instar nymphs (Table 7). Subsequent increase of proteins in the older nymphs was proportionately slower but significant. Thus in 4 day old nymphs TPC variation was from 0.0302 mg/mg to 0.0571 mg/mg and mean value was 0.036 ± 0.0028 mg/mg body weight. The fully developed nymphs gained maximum concentration of proteins and the difference between the minimum and maximum concentration of TPC among the ten nymphs was smaller than that of younger nymphs (Appendix table 4). Therefore, prior to the moulting to the 5th instar the mean TPC in the 4th instar nymphs was 0.039 ± 0.0019 mg/mg body weight (Table 7). Thus during the 4th instar period nymphs gained about 0.023 mg of proteins. As compared to the body weight of the 6 day old nymphs, the percentage of total proteins in these nymphs was 3.9% and as compared to the newly moulted 4th instar nymphs, the ratio of TPC increased in the fully grown 4th instar nymphs was 1: 2.44 (Table 7).

There was a remarkable fall in the TPC during the moulting to the 5th instar nymphs and in the newly moulted 5th instar there was variation from 0.0146 mg/mg to 0.0308 mg/mg and the mean TPC was 0.023 ± 0.0018 mg/mg body weight which was similar to the level of 2 day old 4th instar nymphs. This change was statistically significant when compared with that of the fully developed 4th instar nymphs (6 day old) before the ecdysis (Table 7). It indicated that a greater amount of total proteins was utilised by the instar during the process

Fig. 7. Changes in the total protein concentration per mg weight of the whole body of the 4th and the 5th instar nymphs of _?. circulifer_ related with age, moulting and metamorphosis.

-●- Body weight of the nymphs

-○- Total protein concentration/mg body weight

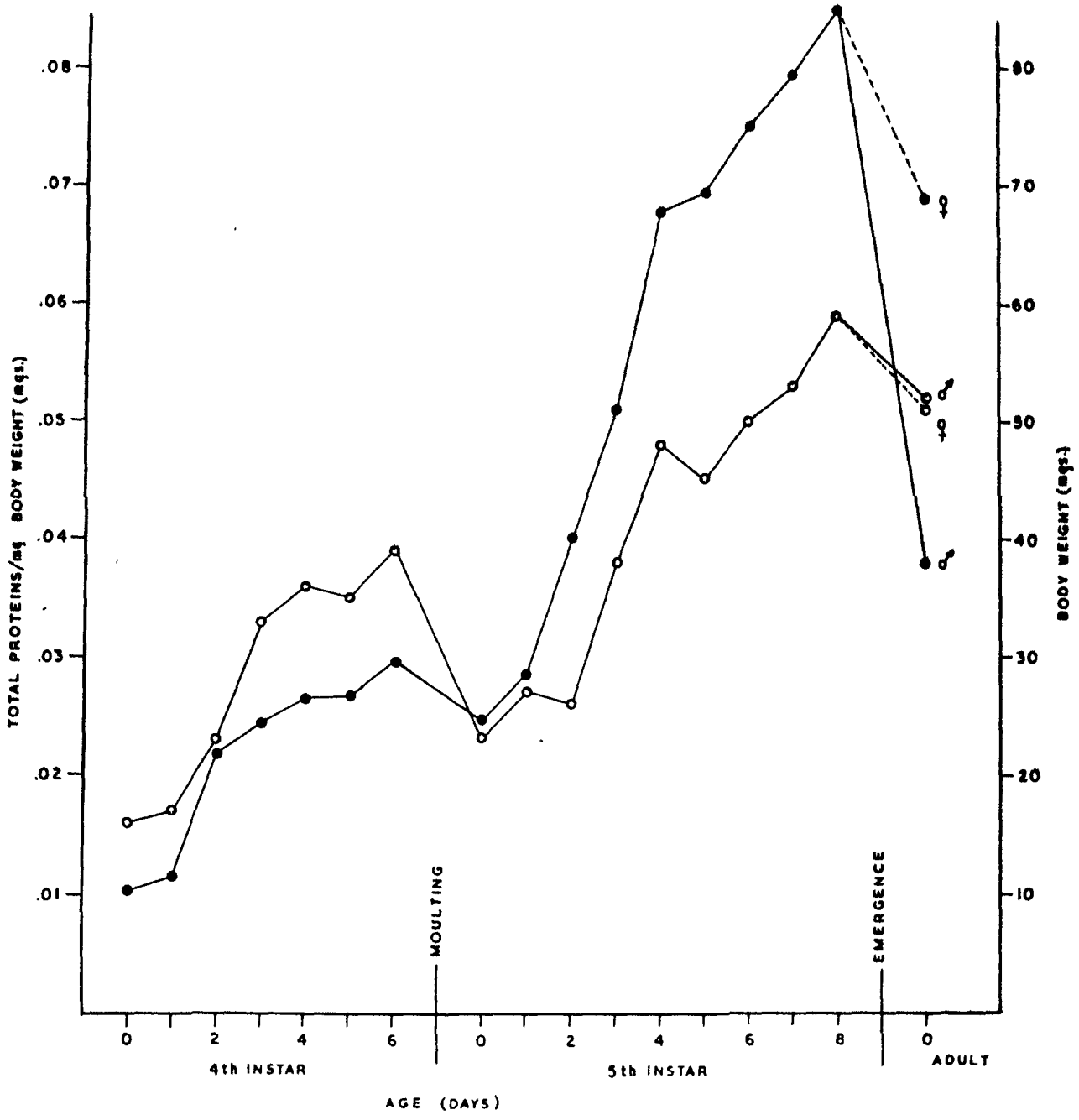

TOTAL PROTEINS/mg BODY WEIGHT (mgs.)
.08
.07
.06
.05
.04
.03
.02
.01
BODY WEIGHT (mgs.)
80
70
60
50
40
30
20
10
♀
♂
♀
♂
MOULTING
EMERGENCE
0
2
4
6
0
2
4
6
8
0
4th INSTAR
5th INSTAR
ADULT
AGE (DAYS)

FIG. 7

of ecdysis. Further, during this instar it was observed that there was a close relationship between the changes in the TPC and the weight of the whole body. The course of increase in the body weight as well as in the TPC/mg body weight seems to be parallel. Further, the rise both in the body weight and the TPC was very sharp in the beginning and later it became gradual (Fig. 7).

From the above observations, it was known that in the 4th instar larva from zero day to the middle of the instar 0.017 mg of the proteins was synthesized whereas 0.006 mg of the proteins was produced in the later period of the instar. Thus, 0.023 mg of the total proteins was synthesized during the whole period of the 4th instar.

The total duration of the 5th instar was eight days. The minimum concentration of proteins in the newly moulted 5th instar nymphs (zero day) was more than half of the maximum TPC in the 4th instar nymphs just before the moulting (Table 7). The loss of the proteins during the ecdysis was slowly compensated during the first two days following moulting to the final instar. Like the 4th instar, in the 5th instar, the increase in the TPC during the first two days following moulting remained slow and insignificant. The mean value of TPC in 1 and 2 day old 5th instar nymphs was 0.027 ± 0.0022 mg/mg body weight and 0.026 ± 0.0014 mg/mg body weight respectively. From the third day onward, increase in the TPC was significant (Table 8). In

Table - 8

Changes in the total protein concentration in the body of the 5th instar nymph of D. circulatus related with age and metamorphosis.

Age (days)	Mean weight of protein/mg of body weight (mg/mg) ± S.E.			't' value	
Zero	0.023	±	0.0010	Control	
1	0.027	±	0.0022	1.754	0.0004
2	0.035	±	0.0014	1.315	0.003
3	0.030	±	0.0014	6.570*	0.045
4	0.048	±	0.0008	10.964*	0.0039
5	0.045	±	0.0016	9.649*	0.010
6	0.050	±	0.0007	11.842*	0.0070
7	0.054	±	0.0028	13.596*	0.010
8	0.059	±	0.0018	15.789*	
Emerged female	0.050	±	0.0047	3.137*	
Emerged male	0.051	±	0.0012	3.194*	

(Table value of 't' at 5% level 18 = 2.101).

3 day old nymphs the loss of proteins was compensated and TPC varied from 0.035 mg/mg to 0.0431 mg/mg (mean, 0.038 ± 0.0014 mg/mg body weight). However, middle aged nymphs of the 5th instar contained almost double concentration (0.048 ± 0.0008 mg/mg) of the newly moulted nymphs (0.023 ± 0.0018 mg/mg). Although there was a slight insignificant TPC fall in the nymphs aged five days as compared to the concentration of the 4 day old nymphs. There was, however, progressive and significant increase in the older nymphs. The mean was 0.050 ± 0.0007 mg/mg body weight and 0.054 ± 0.0023 mg/mg body weight in the nymphs of the 6th and the 7th post-moult day respectively. Maximum TPC was recorded (0.059 ± 0.0018 mg/mg body weight) in the 8 day old nymphs which were fully grown. The ratio of increase in the TPC was 1: 2.6 as compared to the newly moulted 5th instar nymphs. The TPC of the fully grown nymphs was 5.9 percent of the total body weight. The quantity of protein synthesized within the first four days of the 5th instar was 0.025 mg while 0.011 mg protein was synthesized during the last four days. Thus, 0.036 mg of total proteins was synthesized throughout the whole period of larval duration.

Therefore, from the above data it was investigated that the protein concentration in the whole body of the final instar nymphs sharply increased with the advancement of age and weight during the first half of the instar. Later, the rate of increase was slower (Fig. 7). It is also interesting to observe that course of increase in the TPC and the body weight during this instar was almost parallel and similar to the trend observed in the 4th instar nymphs.

Following the metamorphosis of the fully grown nymphs, the total proteins concentration was determined in the males and the females separately. Although the TPC of the newly emerged males and the females did not differ appreciably, these were significantly lighter than the fully grown nymphs (Table 8). The variation of TPC in the newly emerged males was 0.0460 mg/mg to 0.05767 mg/mg whereas in such females it was 0.0449 mg/mg to 0.0537 mg/mg. Such variation range was less than in the fully grown nymphs (Appendix Table 4). During the metamorphosis, the loss of the weight in the males was much greater than that of the newly emerged females. This loss in the TPC and the body weight was significant in both the sexes of the newly emerged adult (Table 4 & 8).

VI. VARIATIONS IN THE TOTAL PROTEINS CONCENTRATION IN THE WHOLE BODY OF *D. CINGULATUS* RELATED WITH AGE AND REPRODUCTION.

In this experiment total proteins concentration of the body was determined in the newly emerged adults of each sex up to a 12 post emergent day (Appendix table 5) in which two reproductive cycles took place at the present controlled conditions.

As recorded in the preceding section, the total proteins concentration related with metamorphosis showed a sharp fall in the newly emerged adults and there was insignificant difference between the males and the females and this difference was again confirmed in the newly emerged adults in the present experiment.

In the males, although the TPC varied in older adults during the twelve days there was no significant changes between the males of the successive age (Table 9). However, the range of variation in the TPC of the males during this period was from 0.0480 mg/mg of the body weight to 0.0585 mg/mg and this difference was statistically significant ($t = 2.584$).

On the other hand, in the females, the changes in the TPC was significant during the corresponding period following the emergence of adults. In the newly emerged females the protein concentration was 0.0505 ± 0.00411 mg/mg of the body

Table - 9

Changes in the total protein concentration in the body of adult *D. cingulatus* related with age and reproduction

Age (days)	MALE				FEMALE			
	Mean of protein/mg body weight (mg/mg)	S.D.	't' value		Mean of protein/mg body weight (mg/mg)	S.D.	't'	
Zero	0.0546	± 0.00502	Control		0.0505	± 0.00411	Control	
1	0.0528	± 0.00585	0.260	1.732	0.0536	± 0.00179	0.687	0.904
2	0.0559	± 0.00537	0.136	0.757	0.0561	± 0.00295	1.103	0.436
3	0.0503	± 0.00200	0.742	0.500	0.0582	± 0.00357	1.409	0.137
4	0.0480	± 0.00349	0.986	1.066	0.0573	± 0.00638	0.994	0.361
5	0.0535	± 0.00318	0.169	0.192	0.0609	± 0.00721	1.529	1.356
6	0.0545	± 0.00417	0.154	0.174	0.0692	± 0.00394	3.274*	1.059
7	0.0535	± 0.00401	0.156	0.193	0.0624	± 0.00530	1.754	2.290*
8	0.0522	± 0.00346	0.328	0.654	0.0573	± 0.00496	1.052	0.424
9	0.0556	± 0.00301	0.171	0.400	0.0600	± 0.00506	1.453	0.152
10	0.0577	± 0.00403	0.468	0.223	0.0616	± 0.00425	1.873	0.371
11	0.0585	± 0.00205	0.742	0.056	0.0631	± 0.00332	2.376*	0.780
12	0.0575	± 0.00431	0.457		0.0503	± 0.00554	1.199	

(Table value of 't' at 5% level 22 (in males) = 2.074 and
" " " " " " " 26 (in females) = 2.056..

Fig. 8. Changes in the total protein concentration per mg wet weight of the whole body of adult *D. simulatus* related with age.

-●- Female body weight

-△- Total protein concentration/mg body weight of the female

-○- Total protein concentration/mg body weight of the males

-▲- Male body weight

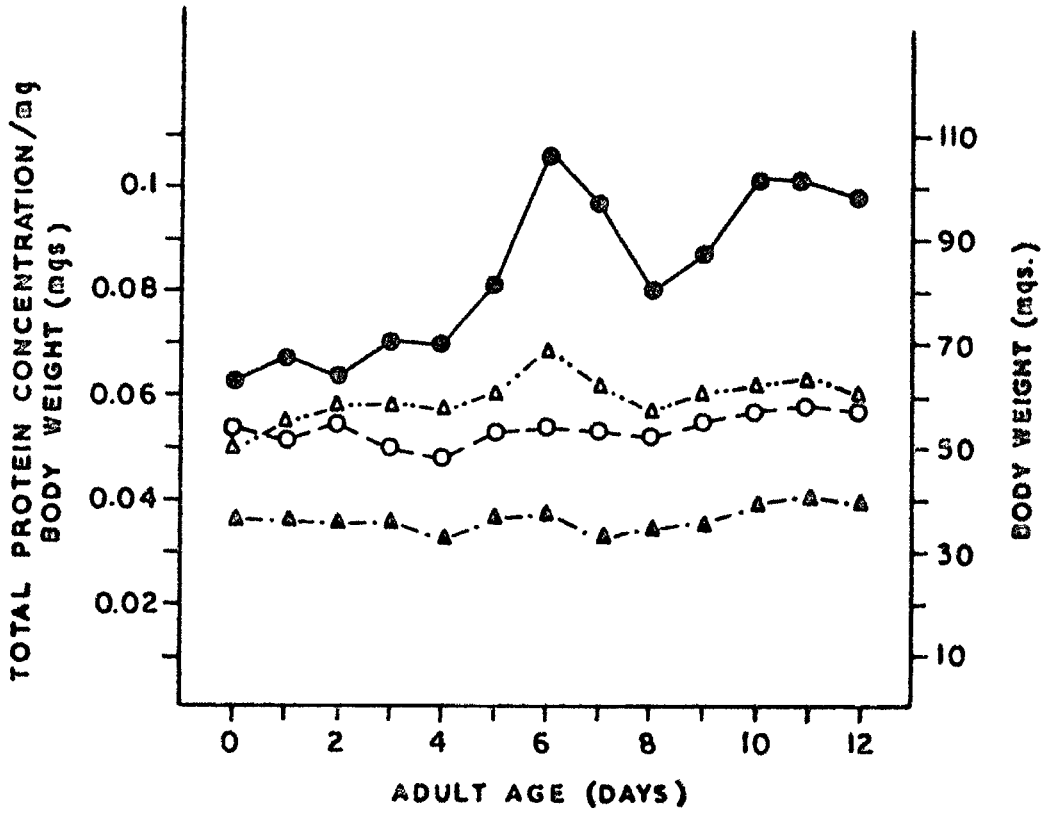

TOTAL PROTEIN CONCENTRATION/mg BODY WEIGHT (mgs)
0.1
0.08
0.06
0.04
0.02
110
90
70
50
30
10
BODY WEIGHT (mgs.)
0
2
4
6
8
10
12
ADULT AGE (DAYS)

FIG. 8

weight and it was the minimum concentration during the period concerned. After five days following emergence there was significant increase (Table 9). This change coincided with the maturation of the ovaries and the peak of the maximal concentration of the total proteins corresponded with the fully matured ovaries on the 6th post-emergent day (0.0692 ± 0.00394 mg/mg body weight) when the females were ready to lay the first batch of eggs. Thus in 7 and 8 day old females the TPC gradually decreased and this change was statistically significant in 8 day old females, as compared to the peak concentration i.e. just before the oviposition. Then following oviposition there was again rising trend in the 9 day old females and a second peak of TPC was observed in the 11 day old females which had fully matured eggs (Fig. 8). This value was significantly higher as compared to that of control, but as compared to the TPC of 9 and 10 day old females it was statistically insignificant increase. Subsequently, there was a sharp fall in the total proteins of 12 day old females due to the oviposition of the second batch of the eggs (Fig. 8). This fall in the TPC was insignificant (Table 9) as compared to that of the matured females of the second reproductive cycle. The value of the second peak of the TPC (0.0631 mg/mg) was lower than that of the first peak (0.0692 mg/mg) but the difference between these two values were insignificant (t = 1.182).

Although there was no significant difference in the TPC of the whole body of the males and the females at emergence

as well as up to 5 days, but in 6 day old females it was significantly higher than that of the males of the corresponding age (t = 2.641). This difference was in the fully matured females and the males. However, in 11 day old adults when the females had the second peak of TPC, the difference between the two sexes was insignificant (t = 1.30) which was also due to slightly higher TPC in the 11 day old males than in the 6 day old males. Thus it can be concluded that in the females, the protein synthesis is much faster after emergence up to the first reproductive cycle than in the second cycle.

VII VARIATIONS IN THE TOTAL PROTEIN CONCENTRATION IN THE OVARIES OF *D. MELANOGASTER* REARED WITH ADULT ENRICHMENT.

The remarkable changes in the total protein concentration (TPC) of the whole body of the females described earlier suggested to estimate the TPC in the ovaries of individual female. Therefore, TPC was recorded per mg of the ovaries of the newly emerged females and in those of subsequent age up to twelve days from emergence. The data on the ovarian TPC of individual female are given in (Appendix table 6) and variations between individuals can be appreciated on their comparison.

The total proteins concentration in the ovarian tissue of the newly emerged females ranges between 0.0083 mg/mg to 0.0166 mg/mg having the mean value 0.0150 ± 0.00093 mg/mg of ovarian tissue which is the minimum concentration during the twelve post-emergent day and this level was about one third of the value of the TPC in the whole body of these females (0.0505 ± 0.00411 mg/mg). Later on, the ovarian proteins gradually increased with the maturation of the oocytes in the ovaries. The enhancement in the ovarian TPC was statistically significant in the females aged 4 to 6 days as compared to the control (in zero age females) (Table 10). But the changes between the ovarian TPC of the females of the adjacent age groups were insignificant. The maximum TPC was recorded in the ovaries of 6 day old females (0.0249 ± 0.00119 mg/mg

Table - 10

Changes in the total protein concentration in the ovaries of *D. circulatus* related with age and reproduction.

Age (days)	Weight of protein/mg of ovarian weight (mg/mg)	S.E.	't' value	
Zero	0.0150	± 0.00093	Control	
1	0.0150	± 0.00014	0.066	0.707
2	0.0170	± 0.00168	1.039	0.500
3	0.0183	± 0.00191	1.549	1.306
4	0.0217	± 0.00150	3.001*	0.201
5	0.0209	± 0.00209	2.506*	1.660
6	0.0249	± 0.00119	6.674*	5.496*
7	0.0161	± 0.00111	0.755	0.583
8	0.0164	± 0.00120	0.900	1.330
9	0.0183	± 0.00135	2.309*	0.549
10	0.0201	± 0.00190	2.430*	0.551
11	0.0213	± 0.00126	4.062*	2.788*
12	0.0160	± 0.00142	0.587	

ovarian tissue) which was somewhat less than double of the minimum concentration in the ovaries of the newly emerged females. Whereas this ovarian concentration was again about one third of the TPC of the whole body of the females of the same age. In the 7 day old females there was a sharp fall in the ovarian TPC and this change was statistically significant as compared to the ovarian TPC of the 6 day old females (Table 10). Similarly the changes in the ovarian TPC of 8 and 9 day old females were also significant. The second peak of ovarian TPC was recorded in the 11 day old females. Although it was significantly higher than that of the newly emerged females (control) it was significantly lower than that of the first peak (t = 2.07) unlike the difference between the two peaks of the TPC of the whole body of the females. It suggested that significantly higher amount of proteins was deposited in the ovaries within the six days following emergence i.e. during the first reproductive cycle as compared to the second reproductive cycle. Thus, after the deposition of the eggs of the first and second batches i.e. on the 7th and 12th post emergent days, the fall in the ovarian TPC was quite significant (t = 5.456 and t = 2.788 respectively) unlike the decrease in the TPC of the whole body of the females of the corresponding age. Further, the TPC in the ovaries of the females following the oviposition of the second batch of the eggs i.e. in the 12 days old females (0.0160 ± 0.00142 mg/mg) was almost equal to that of the newly emerged females (0.0150 ± 0.00093 mg/mg).

Fig. 9. Changes in the total protein concentration/mg weight of the ovaries of adult *A. circulatus* related with age.

- ○ - Total protein concentration/mg ovarian tissue
- ● - Total protein concentration/mg body weight of the female

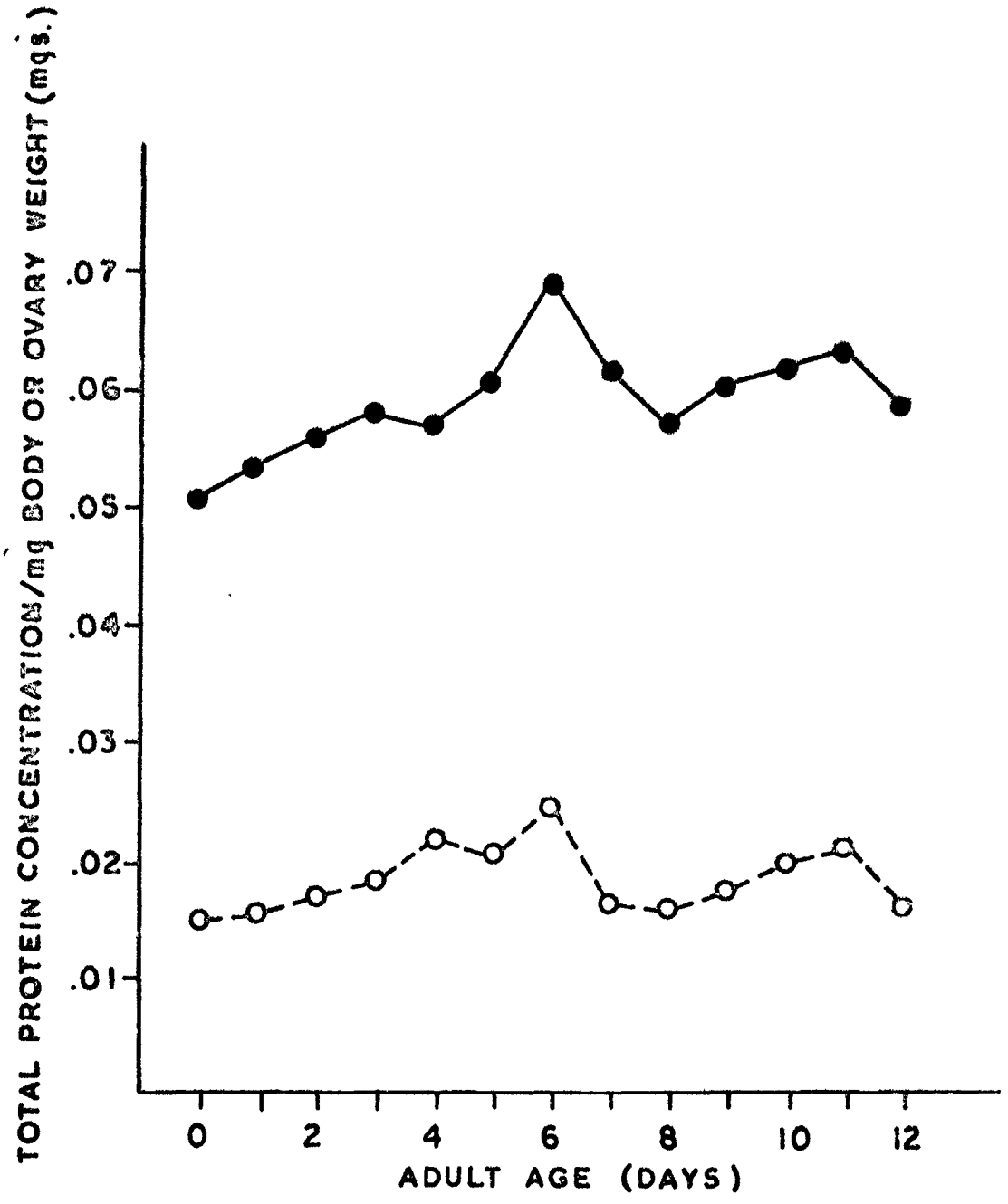

TOTAL PROTEIN CONCENTRATION/mg BODY OR OVARY WEIGHT (mgs.)
.07
.06
.05
.04
.03
.02
.01
0
2
4
6
8
10
12
ADULT AGE (DAYS)

FIG. 9

Thus, the changes in the protein concentration of the ovaries follow the trend similar to that of the whole body of the females of the respective age (Fig. 9). Further, these changes generally correspond with the changes in the total weight of the ovaries (Fig. 6).

VIII ANATOMICAL CHANGES IN THE OVARIES OF *D. CIRCULATUS* RELATED WITH AGE AND REPRODUCTION.

The female *Dysdercus circulatus* has a pair of ovaries. Each ovary has seven ovarioles with terminal filaments. Laterally each ovariole opens into the lateral oviduct of the respective side. The two lateral oviducts open into a common oviduct, the oviductus communis. The oviductus communis opens through the gonopore to the exterior. There is a tubular spermatheca which opens on the common oviduct and its free end is dilated. There is a pair of accessory glands consisting of numerous short tubules arranged in groups. These glands open into the common oviduct near the gonopore. Besides, there is a median accessory gland which is a small tube and opens in the common oviduct very near the spermathecal opening (Plate I).

Each ovariole is telotrophic in nature because the nurse cells remain in the germarium. The developing oocytes descend down in the vitellarium but have cytoplasmic connections with the nurse cells in the early stages of their development. There are two epithelial sheaths covering each ovariole. These sheaths are very distinct in the mature ovarioles where in the region of the large follicles, the outer epithelial sheath is reduced to a thin membrane. Mature follicles are separated from each other by inter follicular cells.

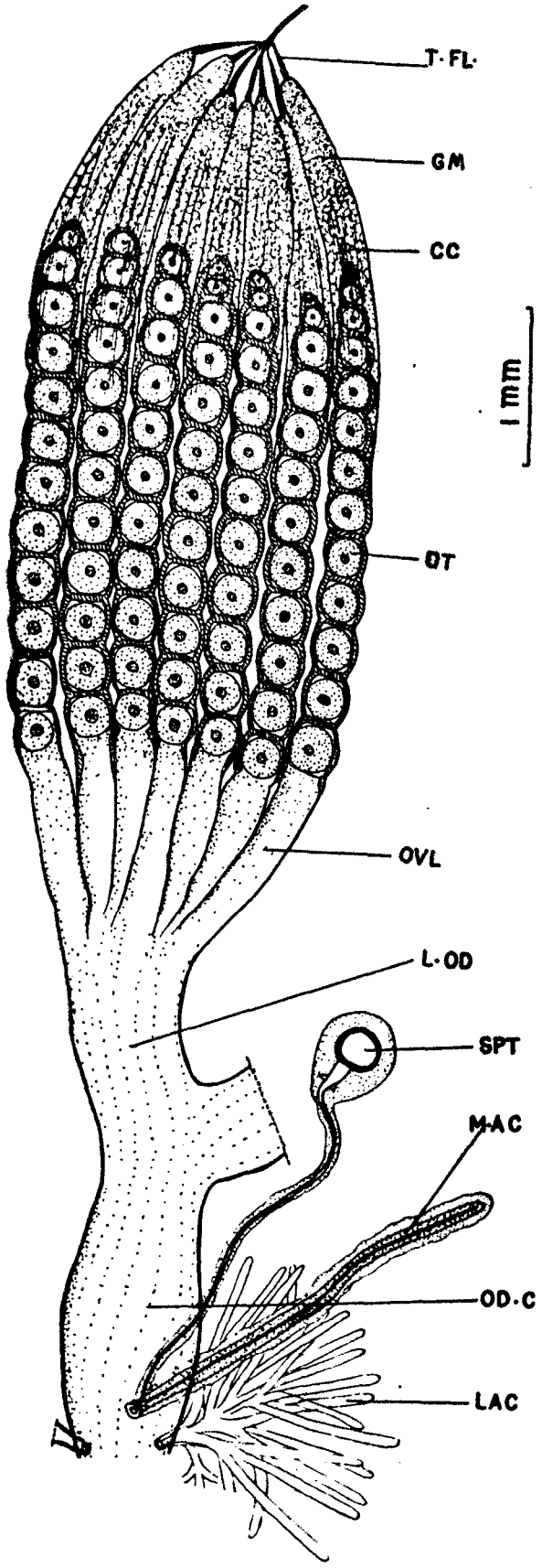
PLATE - I

Internal genitalia of female *D. circulatus* stained in 2% Anilin blue.

Legends:

CC	-	cytoplasmic connections,
GI	-	germarium,
LAG	-	lateral accessory gland,
LOD	-	lateral oviduct,
MAG	-	median accessory gland,
OVD	-	oviductus communis,
OS	-	oocyte,
OVL	-	ovariole,
SP	-	spermatheca,
TFL	-	terminal filament.

PLATE I



The ovaries of the freshly emerged females are composed of very thin ovarioles. There is no anatomical distinction between the germarium and the vitellarium. The size of each ovary is small.

However, the germarium becomes distinct in 1 day old females and it is lanceolate in shape (Plate II, Fig. 1). It is packed with very small cells. Later, progressive development of the germarium takes place. The primordial cells in the germarium appear to increase in size and number in 2 day old females. In the ovaries of the 3 day old females vitellarium becomes distinct and shows beaded appearance due to the growth of the oocytes into ova. Each ovum is enclosed by follicular cells. The ovum lying at the base of the ovariole is the largest in size and there are about 11 or 12 developing ova in each ovariole (Plate II, Fig. 2). At this stage mating also begins and it continues in 4 day old pairs as well. The ovarioles of these females show increased number of follicular chambers with developing ova.

In the 5 day old pairs also mating remains continued and the ovaries of the females become quite large in size. There are about 9 or 10 mature ova in each ovariole. The largest ovum is placed basally near the lateral oviduct. Further, in the 6 day old females, the ovarioles are more matured with a number of ova (Plate II, Fig. 3). Their germarium becomes reduced in size. However, the males still mate with these females.

PLATE - II

Fig. 1. Ovariole of one day old female *D. circulatus*

GI - germarium,

VT - vitellarium,

TFL - terminal filament.

Fig. 2. Ovariole of 3 day old female *D. circulatus*

GM - germarium,

OC - oocyte

TFL - terminal filament,

VT - vitellarium.

Fig. 3. Ovariole of the gravid female (6 day old) *D. circulatus*.

F.C.C - follicular cell,

OLL - outer epithelial sheath,

O - egg

GI - germarium

Fig. 4. Ovariole after the oviposition of the first batch of the eggs (8 day old female).

GI - germarium,

TFL - terminal filament.

PLATE II

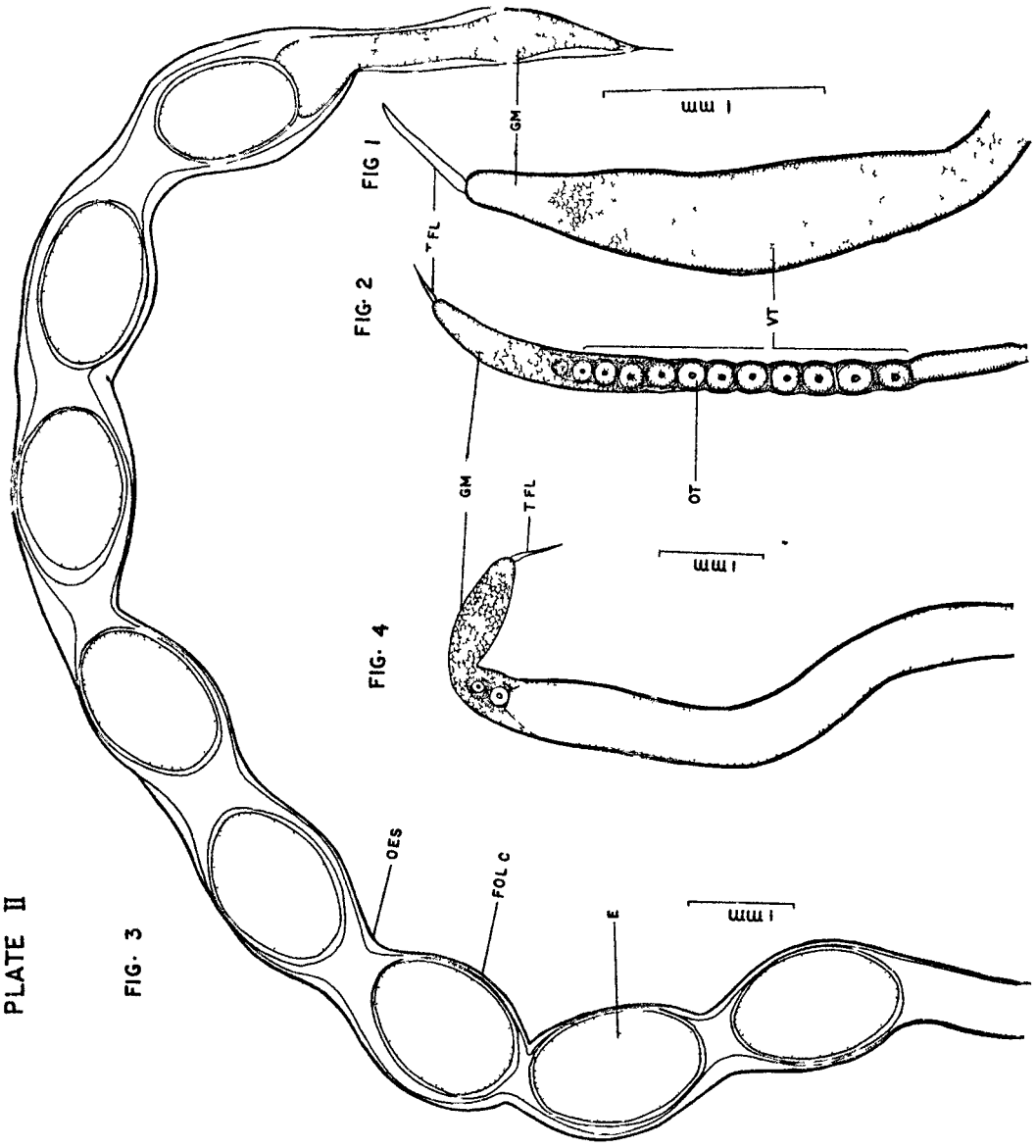


FIG. 3

The first batch of the eggs are laid by the females between 7 and 8 days following emergence. The ovarioles of the 7 day old females in most cases become partially empty whereas the ovarioles of the 8 day old females are empty in all the females (Plate II, Fig. 4). However, mating again starts after sometime following oviposition.

In the 9 day old females, vitellarium again takes the shape of beaded nature and 11 or 12 developing ova are seen enclosed by the follicular cells. Germarium becomes active and packed with white cells. Later, the size of the ovarioles increases more rapidly than in the females before laying the first batch of eggs. Consequently, the 2nd batch of ova mature completely on the 10th or 11th post emergent days. The eggs are completely oviposited by the 12 day old females.

IX THE ELECTROPHORETIC PATTERN OF PROTEINS IN THE OVARIES OF *D. CINGULATUS* RELATED WITH AGE AND REPRODUCTION.

As observed earlier, total protein concentration (TPC) in the ovaries varies in relation to maturation and oviposition of the eggs. It was therefore proposed to study further the changes in different types of proteins in the ovaries of females from their emergence to a period of 12 days. For this purpose separation of different fractions of soluble proteins of the ovaries of the females of different age was done by the Polyacrylamide gel electrophoresis (Devis, 1964). According to the dye-binding capacity, the protein bands were classified as major and minor proteins of the ovaries. Further, the minor category was distinguished as low and feeble fractions. The different fractions were represented by their Rf values (Table 12).

The number of protein bands varied from 9 to 16, depending on the stage of growth and development of the ovaries during 12 days following emergence (Table 11). In the ovaries of the newly emerged females (zero age), 14 protein fractions were detected, three of them were major bands having Rf's 13, 77 and 97 respectively (Table 12) and the protein Rf 97 was highest whereas that of Rf 13 was weakest in concentration (Plate III, & Fig.10). There were eleven minor bands and those with Rf's 16, 32, 46, 67, 74, 84

Table 11

Electrophoretic pattern of protein bands in the ovaries of normal D. cingulatus related with age and reproduction

Age (days)	Condition of the ovary (As described earlier)	Concentration of protein bands			Total number of protein bands
		Major bands	Minor bands		
			Low	Feeble	
Zero	Immature	3	7	4 = 11	14
2	"	5	5	6 = 11	16
4	Maturing	4	2	5 = 7	11
6	Fully matured	3	6	1 = 7	10
8	After the Ist oviposition	3	5	5 = 10	13
10	Fully matured (second time)	3	3	3 = 6	9
12	After the IInd oviposition	3	5	5 = 10	13

Table - 12

Table showing Rf values of different proteins in the ovary of female D. *simulatus* related with age

Rf of bands		Age (days)						
		Zero	2	4	6	8	10	12
Major		-	9	-	-	-	-	-
		13	-	13	-	13	-	13
		-	-	16	16	16	16	16
		-	46	-	-	-	46	-
		-	67	-	-	-	-	-
		-	74	74	74	-	-	-
		77	-	-	-	-	-	-
		97	97	97	97	97	97	97
Minor		-	-	-	-	-	-	-
		-	13	-	-	-	-	-
		16	-	-	-	-	-	-
		-	22	-	22	-	22	22
		-	27	-	27	-	-	27
		32	32	-	-	32	32	-
		-	-	-	-	-	-	37
	Low	46	-	46	46	46	-	-
		-	-	-	48	-	-	-
		-	-	-	-	64	-	-
		67	-	67	67	67	-	67
		74	-	-	-	74	74	74
		84	84	-	84	-	-	-
		87	-	-	-	-	-	-
		-	16	-	-	-	-	-
		22	-	22	-	-	-	-
		-	-	27	-	-	-	-
		-	31	-	-	31	-	-
		-	-	32	32	-	-	32
		-	-	-	-	37	37	-
		41	41	-	-	41	-	41
	Feeble	-	-	-	-	-	-	46
		-	-	-	-	48	48	-
		-	58	-	-	58	-	58
		-	61	-	-	-	-	-
		64	64	64	-	-	-	64
		-	-	-	-	-	67	-
		-	-	84	-	-	-	-
		95	-	-	-	-	-	-
Total no. of protein bands		14	16	11	10	13	9	13

and 87 were low whereas bands, Rf's 22, 41, 64 and 95 were feeble in concentration (Fig. 10). Within the next 2 days, the number of the protein fractions increased to 16. There were five major (Rf's 9, 46, 67, 74 and 97), five low (Rf's 13, 22, 27, 32 & 84) and six feeble bands (Rf's 16, 31, 41, 58, 61 and 64). Only one major protein (Rf 97) was retained in the 2 day old ovaries whereas two other major proteins of zero age ovaries disappeared. A new protein Rf 9 appeared as a major band for the first time although its concentration was comparatively weaker than that of the other major bands within this age. The bands, Rf's 46, 67 and 74 previously observed as low bands, increased in concentration and appeared as major proteins in the 2 day old ovaries. However, a major protein band, Rf 77, observed in the ovaries of the newly emerged females, showed a gradual shift to the band Rf 74 forming a major band of highest concentration (Rf 74) in the ovaries of the 2 day old females (Plate XXI & Fig. 10). Among minor fractions, Rf's 32 and 84 continued as low bands while Rf 13, a major band in the ovaries of the newly emerged females, decreased in concentration and appeared as low band in this age. Further, a feeble fraction Rf 22 in the young ovaries increased in concentration and became a low band. On the other hand, fraction Rf 16 earlier observed as low band became feeble fraction. The protein fractions, Rf's 41 and 64 continued from zero age as feeble bands. Thus in the ovaries of the 2 day old females new protein fractions were Rf 9

(major), Rf 27 (low) and Rf's 31, 58, 61 (feeble) whereas fractions, Rf 77 (major), Rf 87 (low) and Rf 95 (feeble) disappeared.

However, in the 4 day old females, only 11 protein fractions were observed. There were 4 major bands of Rf's 13, 16, 74 & 97 among which band, Rf 74, became weaker in concentration while there was no change in band Rf 97. There was significant change in the bands, Rf's 13 and 16 (minor bands of 2 day old ovaries) as these became more concentrated and formed prominent major bands. The band Rf 9 as major fraction of the ovaries of the two day old females was very short lived. It merged with the band Rf 13 and so the latter increased in concentration. The two major protein bands of the 2 day old ovaries, Rf's 46 & 67 considerably decreased in concentration (Fig. 10). Further, five low protein bands, Rf's 22, 27, 32 & 84 of 2 day old ovaries also reduced in concentration and became the feeble bands in the 4 day old ovaries while band Rf 64 persisted as such. The minor and the feeble bands, Rf's 31, 41, 58 and 61 as observed in the younger ovaries did not continue.

In the fully matured ovaries (in the 6 day old females), 10 protein fractions were detected. It was interesting to record that though the number of the total bands were less than before, the concentration of the major bands were maximum (Plate III and Fig. 10). Three major protein bands (Rf's 16,

PLATE - XII

Photograph of the acrylamide gels showing electrophoretic separation of the ovarian proteins of normal female _D. simulans_ of different age.

RF - relative flow

M - marker

PLATE III

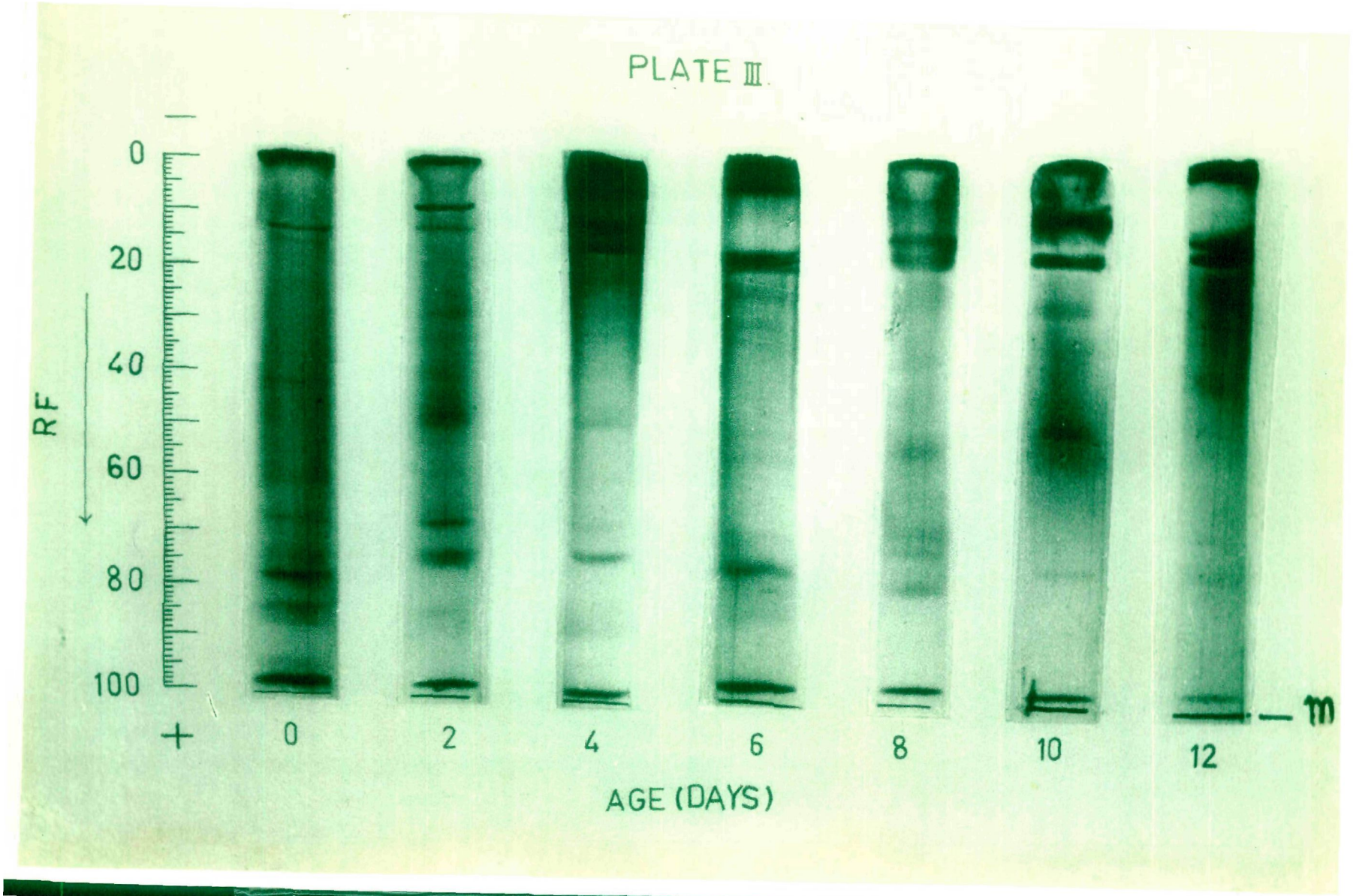


Fig. 10. Traced diagrams of the positions of different protein bands of the normal ovary of *D. cingulatus* related with age (from the electrophoretic separation on acrylamide gel column).

RF - relative flow

CP - cathodic proteins

IP - Isoelectric point

AP - anodic proteins

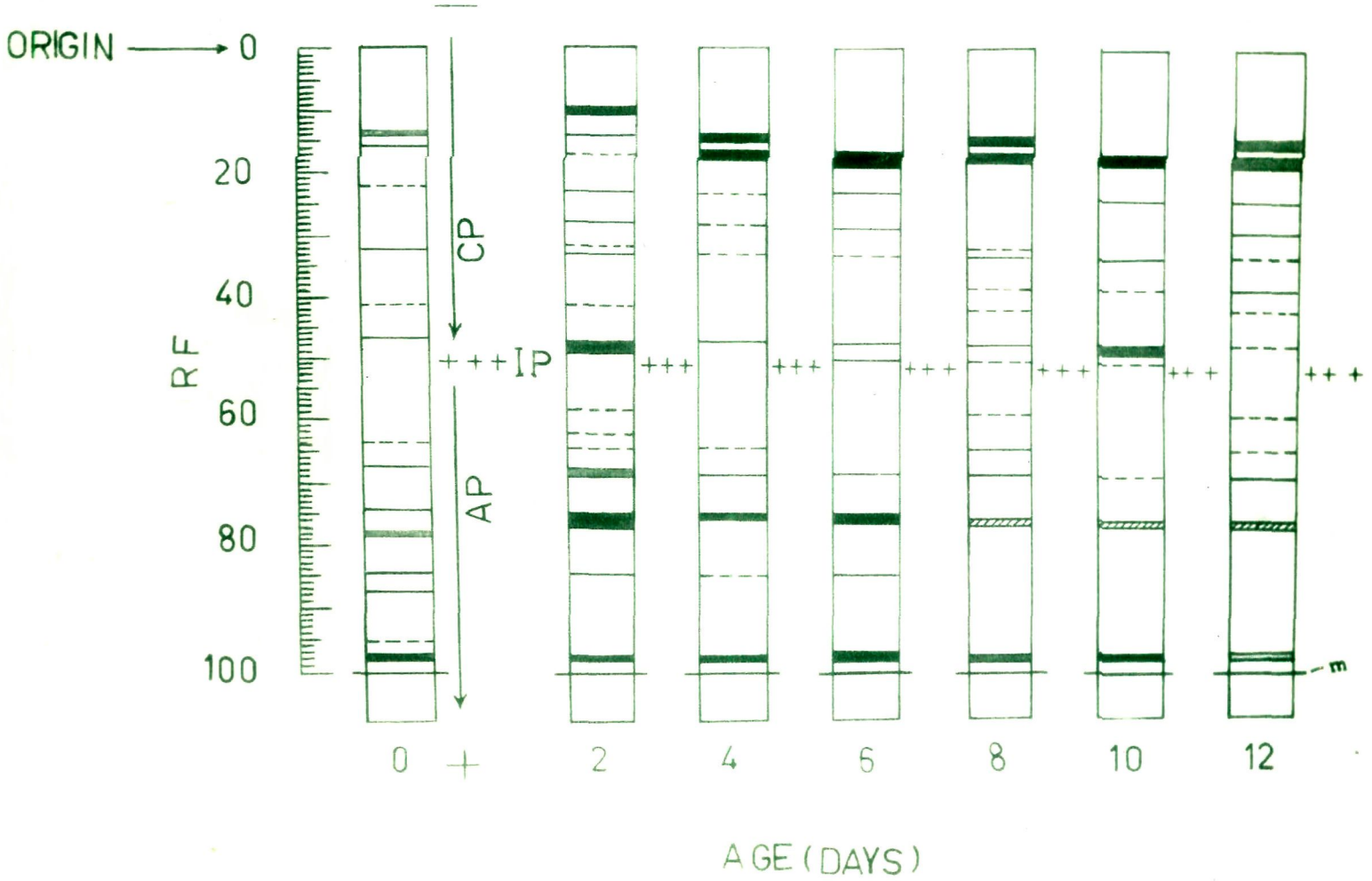
M - marker

▬ - major protein bands

▨ low protein bands

┈┈┈ - feeble protein bands

Fig. 10



74 and 97) were detected and that of band Rf 16 was most voluminous and dark in colour. All these bands continued from the ovaries of the preceding age. Apparently, with the development of the ovaries, bands, Rf's 13 & 16 (of 4 day old ovaries) increased in concentration and eventually fused in the 6 day old ovaries forming one band which had Rf 16 at this stage. The other major bands, Rf's 74 and 97 were also very prominent and dense showing a high concentration of these proteins in the ovaries. As compared to the 4 day old ovaries, the number of low bands also increased. Most of the feeble protein bands of the 4 day old ovaries (Rf's 22, 27 & 84) increased in concentration and appeared as low bands in the matured ovary. The bands, Rf's 46 & 67 continued as low protein bands. However, only one feeble band Rf 32 persisted and a new band Rf 48 of low concentration appeared at this stage of the ovarian development.

Following the oviposition in the 8 day old ovaries, minor bands, Rf 64 (low) and Rf's 31, 41 & 58 (feeble) reappeared together with a new fraction Rf 37 raising the total number of the protein fractions to 13. Other minor protein bands of the 8 day old ovaries which continued from the 6 day old ovaries were Rf's 32, 46, 67, 74 (low) and Rf 48 (feeble). The major protein band, Rf 13, again became prominent from that of Rf 16. The major protein band, Rf 97, persisted although in reduced concentration. However, the major protein fraction Rf 74 was reduced to a low level of concentration (Plate III and Fig. 10).

Qualitatively the concentration of all the major bands was weaker as compared to that of the respective bands in the matured ovaries.

In the ovaries of the 10 day old females, which were again mature for the oviposition of the second batch of eggs, there were only 9 fractions of the proteins. Although the pattern of the protein fractions was almost similar to that of first maturation stage (i.e. 6 day old ovaries), there was qualitative difference between the concentration of major proteins. Among the major protein bands, Rf's 16 and 97 were retained as before. However, band Rf 74 was less prominent and instead Rf 46 became a major fraction. All the major fractions were more dense than the respective fractions in the 8 day old ovaries but weaker as compared to that of the ovaries of the 6 day old females. Again the major band Rf 16 became voluminous and intensified due to the fusion of Rf 13 to Rf 16. The minor band, Rf 32 (low), and the bands, Rf's 37, 43, 67 & 74 (feeble) persisted as such whereas certain minor bands such as Rf's 31, 41 & 64 were utilised by the ovaries. The band Rf 22 reappeared at this stage. The pattern of the protein bands in the ovaries of the 10 day old females showed the disappearance of the minor bands, Rf's 31, 41 & 64. It was interesting to observe that the number of the protein fractions was less in the ovaries matured second time than at the first maturation (Table 11).

Following the oviposition of the second batch of the eggs, the number of the protein fractions again increased to

13 by the reappearance of certain minor bands, Rf's 27, 41, 58 and 64 in the ovaries of 12 day old females. The band Rf 48 completely disappeared. Again, the protein fraction Rf 16 splited into a band Rf 13 and Rf 16 which had lower concentration than before. The protein fraction Rf 97 disappeared although it decreased in concentration. The pattern of the protein fractions in the ovaries of the 12 day old females were somewhat similar to that of the 8 day old females except qualitative difference in the concentration of a few of them.

Since on the basis of the molecular weight, electrically charged molecules of different aminoacids flow in an electric field accordingly, it was also regarded that the protein fractions either charged negatively or positively varied at different stage of the ovarian maturation depending on their appearance in the electrophoretic field. Thus in order to know the charged nature of these proteins the centre of the gel was regarded as isoelectric point (IP) and the protein bands appearing below this point were the positively charged fractions (anodic protein) whereas on the reverse side, the bands indicated the negatively charged proteins (cathodic protein) (Fig. 10).

It was observed that the minimum number of the cathodic proteins were 6 in the ovaries of the newly emerged (zero day) females, it then increased to 9 within two days of the ovarian development. Further, as the maturation of the eggs proceeded

Table - 13

Difference between electrically charged protein fractions in the ovaries of normal *D. cingulatus* related with age

Age (days)	No. of cathodic proteins (-)	No. of anodic proteins (+)	Total number of protein bands
Zero	6	8	14
2	9	7	16
4	6	5	11
6	6	4	10
8	8	5	13
10	6	3	9
12	8	5	13

in the ovarioles, it reduced to the minimum level and remained so up to the fully matured condition of the ovaries in the 6 day old females (Table 13). Following the first oviposition i.e. on the 8th post-emergent day, these cathodic proteins again increased in number. A similar trend of variation was observed in the ovaries of the 10 and the 12 day old females at the time of second maturation and oviposition respectively (Table 13).

On the contrary, the number of positively charged (anodic) proteins were higher than that of the negatively charged (cathodic) proteins in the ovaries of the newly emerged females (Fig. 10). In addition, there was continuous reduction in these proteins as the development of the ovaries proceeded and ultimately reduced to half of the maximum number in the fully matured ovaries of the 6 day old females (Table 13). Further, these proteins increased after the oviposition i.e. on the 8 post emergent day. Minimum number of anodic proteins was recorded in the 10 day old ovaries which matured for the deposition of the second batch of the eggs. The range of variation in the proteins of high molecular weight (cathodic) was 6 to 9 whereas it was 3 to 8 in the proteins of low molecular weight (anodic) during 12 days period. From these observations, it was clear that though the trend of variation in both types of proteins were the same in relation to the age and reproductive cycles, the variation range was greater in anodic proteins, indicating that the anodic proteins were

comparatively much involved in the reproductive cycles (Table 13).

From these observations, it can be inferred that the number of the electrophoretically separable fractions of proteins extensively varied in the ovaries of *D. circulatus* related with age and reproduction. In general, the maximum number of the protein fractions occurred when the ovaries were immature and there was gradual decline in the number of these fractions with the maturation of the ovaries.

X EFFECT OF TOPICAL APPLICATION OF LINDANE ON THE ELECTROPHORETIC PATTERN OF OVARIAN PROTEINS OF D. CINGULATUS RELATED WITH AGE AND REPRODUCTION.

As earlier mentioned the electrophoretic pattern of proteins of the normal ovary of D. cingulatus revealed a number of protein fractions which decreased in number in relation to the maturation of the eggs. It further indicated that certain proteins are used up during the maturation of the eggs. This formed a basis to investigate the effect of the insecticides on the proteins of the ovaries. For this purpose, a sublethal dose of technical Lindane (0.0125%, LD_{50}), a chlorinated hydrocarbon, was topically applied on the last instar nymphs of D. cingulatus and protein fractions were separated in the homogenate of the ovaries of the females of different age (affected females), following emergence.

In the ovaries of the newly emerged females (zero age) from the treated nymphs the number of the protein fractions was similar (14) to that of the normal ovaries of the corresponding age. But the pattern and the concentration of these fractions varied as compared to that of the normal ovaries of this age. The protein bands, Rf's 81, 12, 20, 48, 62 and 93 appear to be new bands in the ovaries of the affected females. However, they had very close placing with Rf's 77, 13, 22, 46,

Table - 14

Electrophoretic pattern of protein bands in the ovaries of *D. cingulatus* of different age treated with Lindane (nymphal treatment)

Age (days)	Concentration of protein bands			Total number of protein bands
	Major bands	Minor bands		
		Low	Feeble	
Zero	3	3	8 = 11	14
2	4	5	3 = 8	12
4	2	3	4 = 7	9
6	2	2	3 = 5	7
8	1	5	6 = 11	12
10	3	5	2 = 7	10
12	1	4	4 = 8	9

Table - 15

Table showing Rf values of different proteins in the ovaries of D. circulatus of different age treated with Lindane, (nymphal treatment)

Rf of band		Age (days)						
		Zero	2	4	6	8	10	12
Major		-	-	-	-	-	6	-
		9	-	9	-	9	9	-
		-	-	-	-	-	-	-
		-	12	-	-	-	12	-
		-	-	-	16	-	-	16
		-	-	-	20	-	-	-
		-	67	-	-	-	-	-
		74	74	-	-	-	-	-
		81	-	-	-	-	-	-
		-	97	97	-	-	-	-
Minor	Low	-	9	-	-	-	-	-
		12	-	-	-	12	-	-
		13	-	-	-	-	-	-
		-	14	-	-	-	-	-
		-	-	16	-	-	-	-
		-	20	-	-	-	20	-
		-	-	-	27	-	-	27
		-	32	-	-	32	-	-
		41	-	-	-	-	-	-
		-	48	-	-	-	48	48
		-	-	-	-	-	62	-
		-	-	-	-	67	67	67
		-	-	74	-	74	-	-
		-	-	81	-	-	-	-
		-	-	-	97	97	97	97
	Feeble	-	-	-	-	6	-	-
		20	-	-	-	20	-	20
		-	22	22	-	-	-	-
		-	27	27	-	-	27	-
		32	-	-	-	-	-	32
		-	-	-	-	41	41	-
		48	-	-	48	48	-	-
		58	-	-	-	-	-	-
		62	-	62	-	62	-	62
		67	-	67	67	-	-	-
		-	-	-	74	-	-	74
		-	81	-	-	81	-	-
		93	-	-	-	-	-	-
		97	-	-	-	-	-	-
Total no. of protein bands		14	12	9	7	12	10	9

64, 95 of the normal ovaries. But bands, Rf's 9 and 58 appeared earlier than that of the normal ovaries in which they were observed in 2 day old females (Fig. 10 and Fig. 11). There were three major bands, Rf's 9, 74 and 81 among which band, Rf 81 was qualitatively most concentrated while Rf 74 was weakest. All these three major fractions were qualitatively weaker in concentration than the other three major bands of the normal ovaries of the corresponding age. There were only three low bands, Rf's 12, 13 and 41 but eight feeble protein bands, Rf's 20, 32, 48, 58, 62, 67, 93 and 97. The most interesting feature was that bands, Rf's 13 and 97 which were extremely prominent in the normal ovaries of this age, considerably decreased in concentration in the affected ovaries and appeared as low and feeble bands respectively. Thus, in the ovaries of the newly emerged females from treated nymphs, as compared to the protein bands of the normal ovaries, the major bands were weaker, the number of low bands reduced whereas that of feeble bands increased, indicating a remarkable decrease in protein concentration on the whole (Plate III and IV).

Further, unlike the normal ovaries, within next two days, the number of the protein fractions decreased to 12 (Fig. 13). But there were 4 major bands, Rf's 12, 67, 74 and 97 among which the band Rf 74 persisted while those of Rf's 12, 67 and 97 turned to the major bands from the low and the feeble fractions of the affected ovaries of the newly emerged females (Table 15). Though the major bands of Rf's 67, 74 and 97 were

comron with the normal ovaries of this age, these were qualitatively weaker in concentration than those of the normal ovaries. The major bands of the ovarian proteins of the newly emerged females Rf's 9 and 91 considerably decreased and formed low and feeble bands respectively at this age. The low fraction Rf 13 of the ovaries of the newly emerged females continued as low fraction Rf 14 in the 2 day old females. The other three low fractions of this age Rf's 20, 32 and 48 earlier appeared as feeble fractions. The new feeble fractions in the ovaries of the 2 day old affected females were Rf's 22 and 27 whereas the fractions, Rf's 41 (low) and 58, 62 and 93 (feeble) disappeared. Thus two minor fractions were lost reducing the total number of protein bands to 12 (Table 14).

Further, in the 4 day old ovaries, the number of the protein fractions reduced particularly in the category of major and low fractions and the total number was only 9. There were only two major bands of Rf's 9 and 97 in which Rf 9 previously appeared as low band in the 2 day old ovaries. However, in the present age this band increased in concentration and fused with the major band Rf 12 and became more voluminous and concentrated band of this age. The previously detected major band Rf 97 continued as more dense major band of this age. The protein bands, Rf's 67 and 74 observed as major bands in the 2 day old ovaries diminished in concentration and became feeble and low protein fractions respectively

in the 4 day old ovaries. The low protein band Rf 14 of the 2 day old ovaries apparently showed a gradual shift to Rf 16 of the 4 day old ovaries and remained low in concentration. Among feeble fractions, Rf's 22 and 27 persisted as such whereas band Rf 62 reappeared. However, the fractions, Rf's 20, 32 and 48 (low) of the 2 day old ovaries disappeared. Thus, as compared to the normal pattern of the ovarian proteins, it was recorded that two major proteins were lost in number in the affected ovaries of the 4 day old females (Fig. 13).

In the ovaries of the 6 day old affected females (i.e. the stage corresponding to matured ovaries of the normal females) the protein fractions decreased to 7 as compared to the normal 10 bands of the proteins in this age (Table 11 and 14). In the ovaries of the affected females of this age there were only two major protein fractions with Rf's 16 and 20. The protein as Rf 16 detected as low band in the 4 day old ovaries, increased in concentration and the prominent major band Rf 9 of the 4 day old ovaries eventually merged into it forming a broad and prominent major band Rf 16 in the 6 day old ovaries. The second major protein Rf 20 in this age was less concentrated than Rf 16 and it transformed from a minor band in the younger ovaries whereas the major protein fraction Rf 97 earlier present in the ovaries considerably decreased in concentration and became a low band protein at this age. On the other hand, the protein Rf 27 which was earlier feeble

band appeared as low band. The three feeble protein fractions, Rf's 48, 67 and 74 were detected and out of these only Rf 67 continued as feeble from the preceding age whereas the low fraction Rf 74 of the 4 day old ovaries reduced to feeble band of the 6 day old ovaries. The feeble protein fraction Rf 48 of the ovaries of the newly emerged females reappeared in the 6 day old ovaries. The feeble fractions, Rf's 22 and 62 of the 4 day old ovaries completely disappeared in the matured ovaries.

On the 8th day following emergence i.e. the time coinciding the first oviposition, the ovaries of the affected females showed an increase in the number of some minor protein fractions raising the total number to 12. The electrophoretic pattern of the protein fractions of the 8 day old ovaries of the affected females showed remarkable reduction in the number of major proteins as compared to that of normal ovaries of the corresponding age, resulting in only one major fraction Rf 9. Apparently it showed that the most prominent and broad band Rf 16 of the 6 day old ovaries decreased in concentration during a couple of days and splited into a thin and less concentrated major protein band Rf 9 and low band Rf 12. The less concentrated major band Rf 20 of the 6 day old ovaries considerably decreased and persisted as feeble band in the 8 day old ovaries. The other low bands observed were Rf's 32, 67, 74 and 97 among which Rf 32 reappeared as it was earlier utilized. Whereas bands, Rf's 67, 74 and 97 continued from the preceding age, the protein

PLATE - XV

Photograph of the acrylamide gels showing electrophoretic separation of the ovarian proteins of female *D. cingulatus* (affected with Endemo) of different age.

RP - relative flow

M - marker

PLATE Ⅳ.

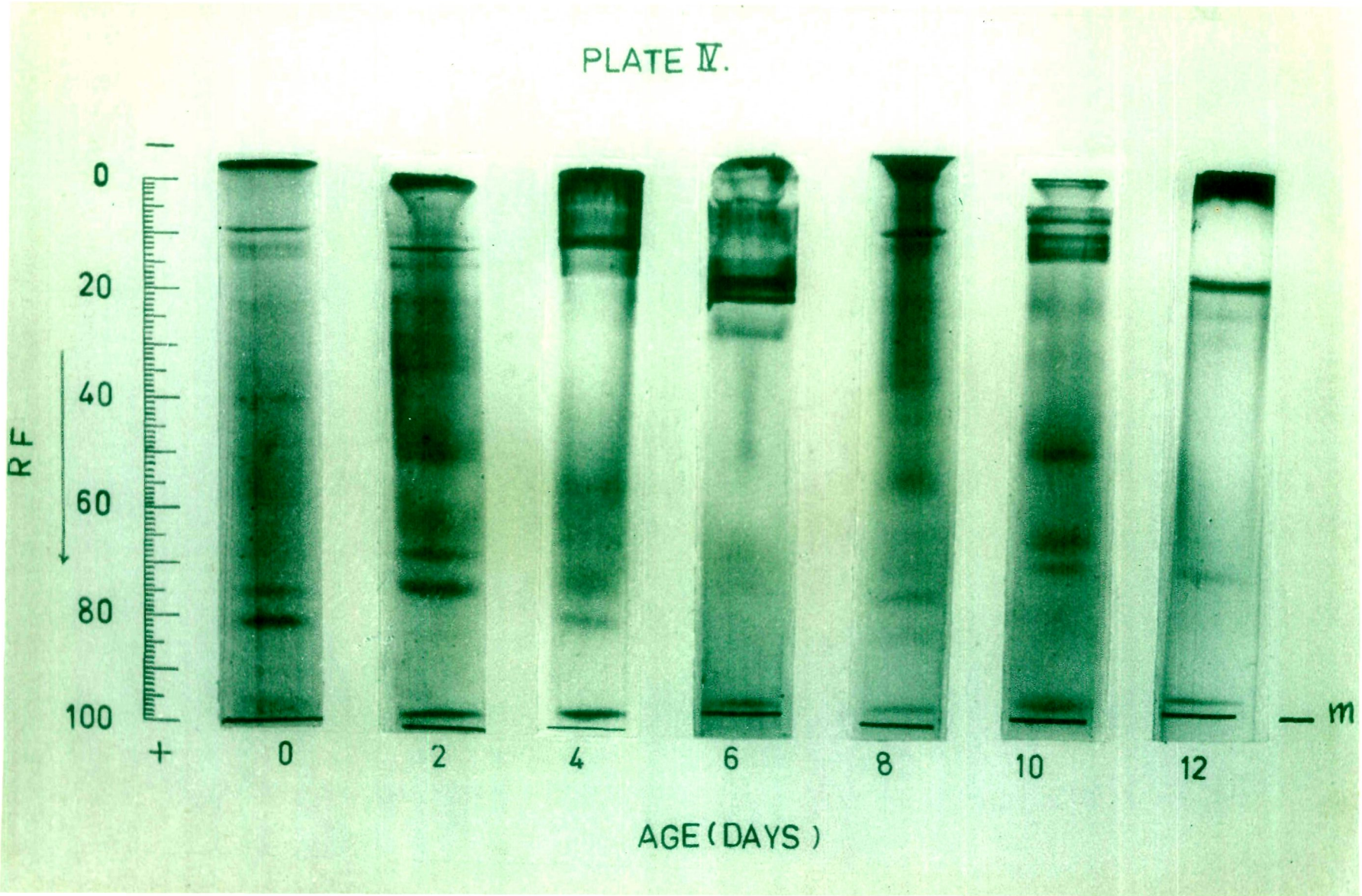


Fig. 11. Traced diagrams of the positions of different protein bands of the ovary of lindane affected female *D. circulatus* related with age (from the electrophoretic separation on acrylamide gel column).

RF - relative flow

CP - cathodic proteins

IP - isoelectric point

AP - anodic proteins

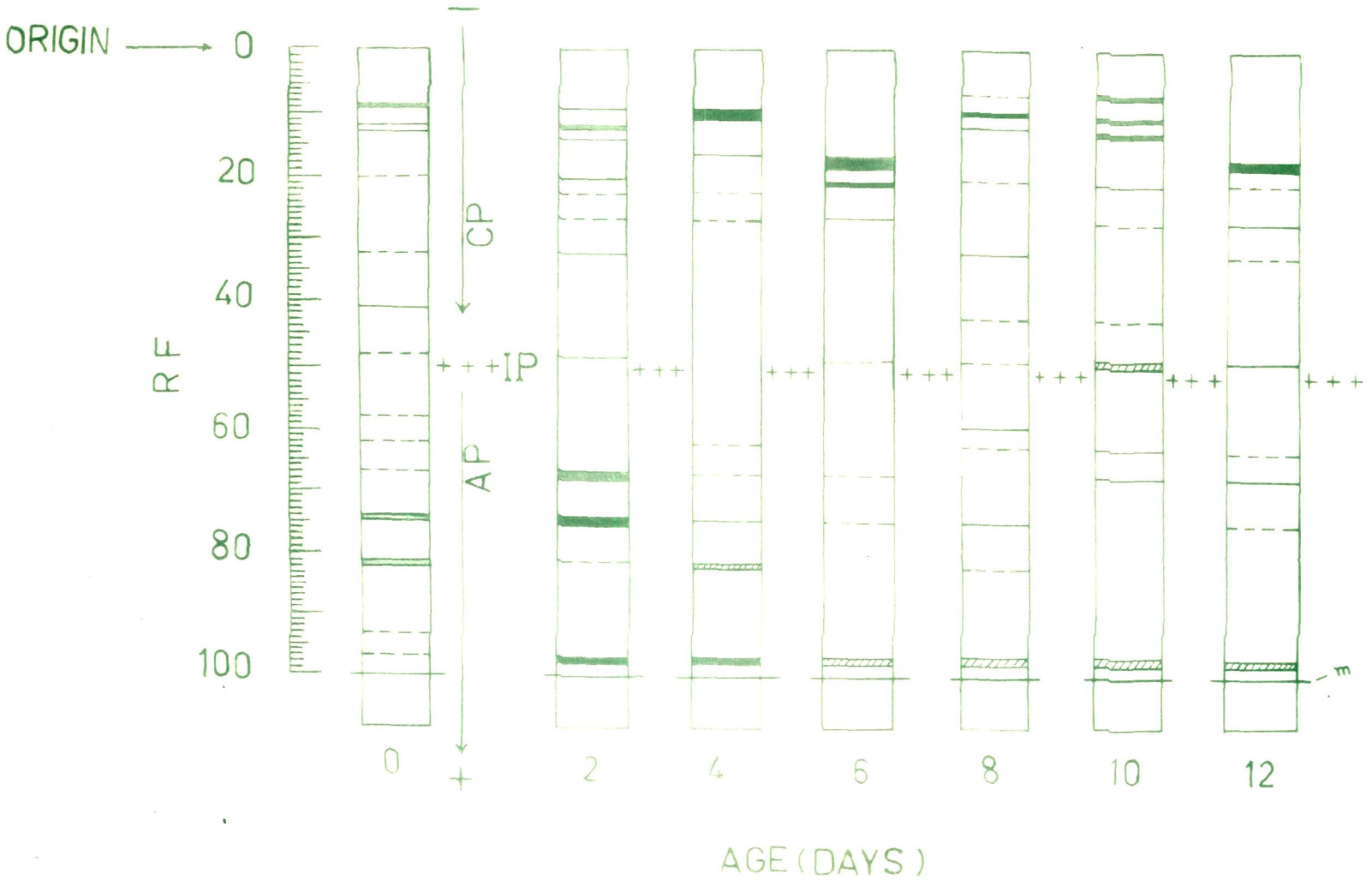
M - marker

▬ - major protein bands

{▨ low protein bands

------ - feeble protein bands

Fig. 11



Rf 27 (low) completely disappeared. There were six feeble protein bands, Rf's 6, 20, 41, 48, 62 and 81. The fraction Rf 48 persisted as such from the previous age while proteins, Rf's 20, 41, 62 and 81 reappeared raising the total number of the protein fractions of the 8 day old ovaries. A new feeble fraction Rf 6 which was not observed previously at any age, appeared at this stage. Although the number of the total protein fractions in the affected ovaries of the 8 day old females did not differ much from that of the normal ovaries of the respective age, the higher concentration in the protein fractions of the latter can be qualitatively appreciated (Plate III and Plate IV).

In the ten day old affected ovaries, again the number of the total protein fractions reduced to 10. On the other hand, the number of the major bands increased while that of minor bands decreased. Further, the pattern qualitatively indicated higher concentration of the proteins as compared to that of the previous age. There were three major fractions, Rf's 6, 9 and 12 indicating the carry over of the major band Rf 9 from the 8 day old ovaries whereas the low fractions, Rf's 6 and 12 of the previous age increased in concentration and appeared as major fractions in the ten day old ovaries. However, these major protein fractions were not very prominent. There were five low bands, Rf's 20, 48, 62, 67 and 97 among which Rf's 67 and 97 continued as such from the 8 day old ovaries while Rf's 20, 48, and 62 previously observed as feeble bands intensified as low

band in this age. There were only two feeble bands Rf's 27 and 41 in which the latter persisted as such from the previous age while the former reappeared. The protein fractions, Rf's 32 and 74 (low) and 81 (feeble) disappeared from these ovaries. Further, there was a remarkable decrease in the concentration of the major bands of the ten day old ovaries as compared to the normal ovaries of the respective age (Plate III and Plate IV).

Unlike normal ovaries, the total number of proteins in the 12 day old ovaries of the affected females reduced to 9 (Fig. 13) having one major (Rf 16), four low (Rf's 27, 48, 67 and 97) and four feeble (Rf's 20, 32, 62 and 74) protein fractions. The three less prominent major bands (Rf's 6, 9 and 12) of the 10 day old ovaries apparently fused forming a prominent and more voluminous band Rf 16 of the 12 day old affected ovaries. The feeble fraction Rf 27 of the previous age increased in concentration and became low fraction whereas other low fractions (Rf's 46, 67 and 97) continued unchanged. The low fractions of the 10 day old ovaries, Rf's 20 and 62 decreased in concentration and appeared as feeble fractions. The only feeble fraction lost was Rf 41 while new feeble fractions, Rf's 32 and 74 reappeared, suggesting that though the total number of the protein fractions decreased, their concentrations comparatively increased from the previous age. The range of variation in the ovarian protein fractions of the affected females was 7 to 14 during the 12 days period whereas that of the normal ovaries was 9 to 16.

Table - 16

Difference between electrically charged protein fractions in the ovaries of D. cingulatus of different age treated with Lindane (nymphal treatment)

Age (days)	No. of cathodic proteins (-)	No. of anodic proteins (+)	Total number of protein bands
Zero	7	7	14
2	8	4	12
4	4	5	9
6	4	3	7
8	6	6	12
10	7	3	10
12	5	4	9

In the ovaries of the newly emerged adults (zero day) topically treated with Lindane (LD_{50}) at the nymphal stage, cathodic and anodic proteins were equal in number i.e. 7 each. Thereafter, the anodic proteins started decreasing and its number reduced to less than half of the maximum number up to the 6 day of the ovarian development (Table 16). On the other hand, cathodic proteins of the ovaries remained almost unchanged within two days following emergence. Subsequently, these also decreased to a minimum level (4) in the 6 day old ovaries. Later, within a couple of days, there was again increase in both types of the proteins reaching almost to the equal number (Table 16). However, in the 10 day old ovaries, there was a decline in anodic proteins while enhancement in the cathodic proteins (Table 16). Afterwards, the cathodic proteins decreased in number. The range of variation in the cathodic and the anodic proteins was 4 to 8 and 3 to 7 respectively within 12 days period.

On comparison with the electrophoretic pattern of the normal ovaries it was found that in the ovaries of the affected females, during the first half of the 12 days duration, the trend of the variation in both types of proteins was similar to that of the normal ovaries (Fig. 14). But it differs in the later half of this period and this change was more pronounced in the negatively charged proteins (cathodic) than that of the positively charged proteins (Anodic).

XI EFFECT OF TOPICAL APPLICATION OF PARATHION ON THE ELECTROPHORETIC PATTERN OF THE OVARIAN PROTEINS OF *D. CINGULATUS* RELATED WITH AGE AND REPRODUCTION.

As a representative of the organophosphorus insecticides, Parathion (Methyl Parathion Tech, 80%) was selected to compare with the effect of the chlorinated hydrocarbon, Lindane on the electrophoretic pattern of the ovarian proteins of *D. cingulatus*. For this purpose a sub-lethal dose of technical parathion (0.0078%, LD_{50}) was applied on the 5th instar nymphs in the same manner as that of Lindane. The variations in the protein fractions of the ovaries of the affected females of different age were studied.

In the ovaries of the newly emerged females from the treated nymphs, the number of the protein fractions was similar to that of the ovarian proteins of the unaffected females but the electrophoretic pattern and the concentration of different proteins varied (Table 11 & 17, Fig. 10 and Fig. 12). In the ovaries of the newly emerged affected females, a slow-moving band Rf 6 appeared as new fraction which was not found at any stage in the normal ovaries of the corresponding age. Further, in the ovaries of the affected females, the protein bands, Rf's 12, 51, 56, 71, 81 and 86 had close placing with Rf's 13, 46, 58, 74, 84 and 87 of the normal ovaries of the unaffected females. However, the protein bands, Rf's 9, 36 and 37 appeared earlier

Table - 17

Electrophoretic pattern of protein bands in ovaries of D. circulatus of different age treated with Parathion (nymphal treatment)

Age (days)	Concentration of protein bands				Total number of protein bands
	Major bands	Minor bands			
		Low	Feeble		
Zero	3	5	6 =	11	14
2	4	5	6 =	11	15
4	3	1	8 =	9	12
6	2	3	4 =	7	9
8	3	5	9 =	14	17
10	3	2	8 =	10	13
12	2	3	6 =	9	11

Table - 18

Table showing Rf values of different proteins in the ovaries of D. cingulatus of different age treated with Parathion (nymphal treatment)

Rf of band		Age (Days)						
		Zero	2	4	6	8	10	12
Major		6	-	-	-	-	-	-
		9	-	-	9	-	-	9
		-	10	-	-	-	-	-
		12	-	12	-	12	12	12
		-	-	16	-	16	16	-
		-	56	-	-	-	-	-
		-	77	-	-	-	-	-
		-	97	97	97	97	97	-
Minor	Low	-	6	-	-	6	-	-
		1-	13	-	-	-	-	-
		-	22	-	22	22	-	-
		37	-	-	-	-	-	-
		41	41	-	-	-	-	-
		-	-	-	-	-	-	46
		51	-	-	-	-	51	-
		56	-	-	56	-	-	-
		-	-	-	-	67	-	-
		-	-	-	71	71	71	71
		-	-	77	-	-	-	-
		-	86	-	-	86	-	-
		97	-	-	-	-	-	97
	Feeble	-	-	6	6	-	-	-
		22	-	22	-	-	22	22
		-	24	-	-	24	-	24
		-	-	27	27	27	27	27
		-	-	-	-	29	-	-
		-	-	-	-	32	32	32
		-	37	-	-	37	37	-
		-	46	-	-	-	46	-
		-	-	-	-	48	-	-
		-	51	51	-	51	-	-
		-	-	-	-	56	56	-
		64	-	64	-	64	-	-
		-	67	67	67	-	67	67
		71	-	71	-	-	-	-
		74	74	-	-	-	-	-
		-	-	-	77	-	-	-
		81	-	-	-	-	-	-
		86	-	86	-	-	86	86
Total no. of protein bands		14	15	12	9	17	13	11

than that of the normal ovaries in which they were detected in 2 and 8 day old females respectively (Table 12 & 18).

Like the normal ovaries of the unaffected females, there were three major protein fractions with Rf's 6, 9 and 12 in the ovaries of the affected females of the same age but qualitatively quite weaker in concentration as compared to the major ovarian proteins of the normal females of the corresponding age (Plate III and V). The protein bands, Rf's 13 and 97 which existed as strong major bands in the ovaries of the normal females, considerably decreased and appeared as Rf 12, a less prominent major band and a low fraction Rf 97 respectively in the affected ovaries. Further, there were five low (Rf's 37, 41, 51, 56 and 97) and six feeble (Rf's 22, 64, 71, 74, 81 and 86) protein bands making the total number of bands 14 in the ovaries of the newly emerged affected females. But qualitatively, the concentration of the protein fractions of the affected ovaries considerably decreased as compared to that of the normal ovarian protein fractions of the corresponding age (Fig. 10 and Fig. 12).

Like the number of the ovarian proteins of the normal females, within a couple of days, there was increase in the protein fractions of the affected females making the total number 15 which consisted of four major bands, Rf's 10, 56, 77 and 97. The major band Rf 9 of the ovaries of the newly emerged females increased in concentration and persisted as

Rf 10 whereas the fractions, Rf's 56 and 97 were the progressively transformed major fractions from the respective low fractions of the younger ovaries. The major fraction Rf 77 appeared as a new band although it was a normal band in the normal ovaries of the newly emerged females (Fig. 10). Among the four major fractions, band Rf 97 was qualitatively strongest in concentration. The major bands, Rf's 6 and 12 of the younger ovaries reduced to low fractions with Rf's 6 and 13 in the 2 day old affected ovaries. The low fractions, Rf's 22 and 66 appeared as transformed from the respective feeble fractions of the younger ovaries whereas Rf 41 persisted as such. The low fractions, Rf's 37 and 51 observed before, decreased in concentration and turned to feeble fractions in the 2 day old affected ovaries. However, the bands, Rf's 24, 46 and 67 were new additions while that of Rf 74 persisted from the newly emerged affected females. The three feeble protein bands, Rf's 64, 71 and 81 of the previous age disappeared. In general there was qualitative decrease in the protein fractions of the 2 day old ovaries of the treated females as compared to that of the normal ovaries of the same age (Fig. 10 and Fig. 12).

Within four days following emergence, there was a decline in the number of the ovarian proteins particularly that of the major and the low fractions and the total number was reduced to 12. There were three major protein bands, Rf's 12, 16 and 97. Apparently the major band Rf 10 of the previous age shifted and appeared as more prominent major band

PLATE - V

Photograph of the acrylamide gels showing electrophoretic separation of the ovarian proteins of female *C. cingulatus* (affected with Parathion) of different age.

RF - relative flow

m - marker

PLATE V.

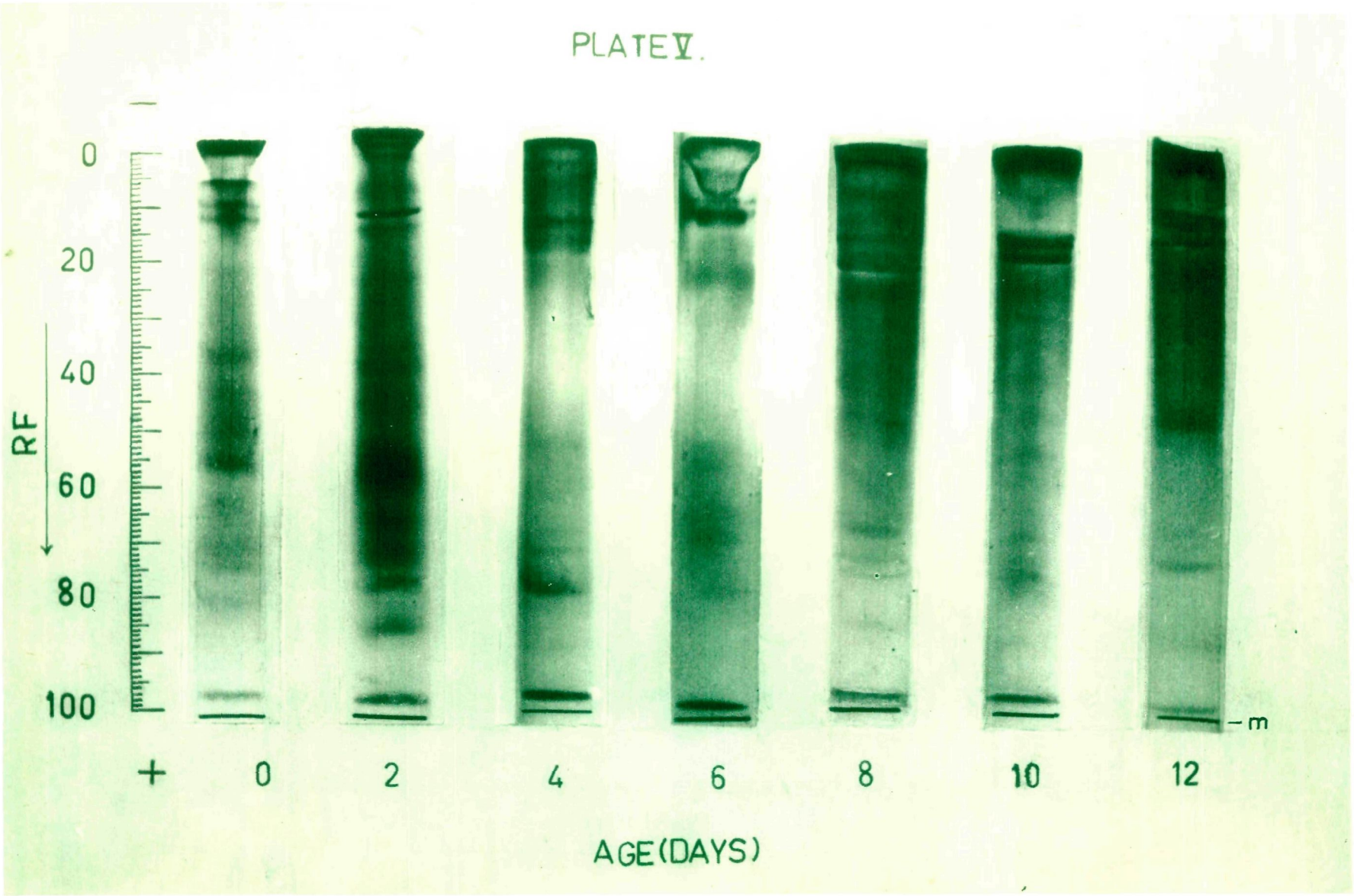


Fig. 12. Traced diagrams of the positions of different protein bands of the ovary of parathion affected female D. cingulatus related with age (from the electrophoretic separation on acrylamide gel column).

RF - relative flow

CP - cathodic proteins

IP - isoelectric point

AP - anodic proteins

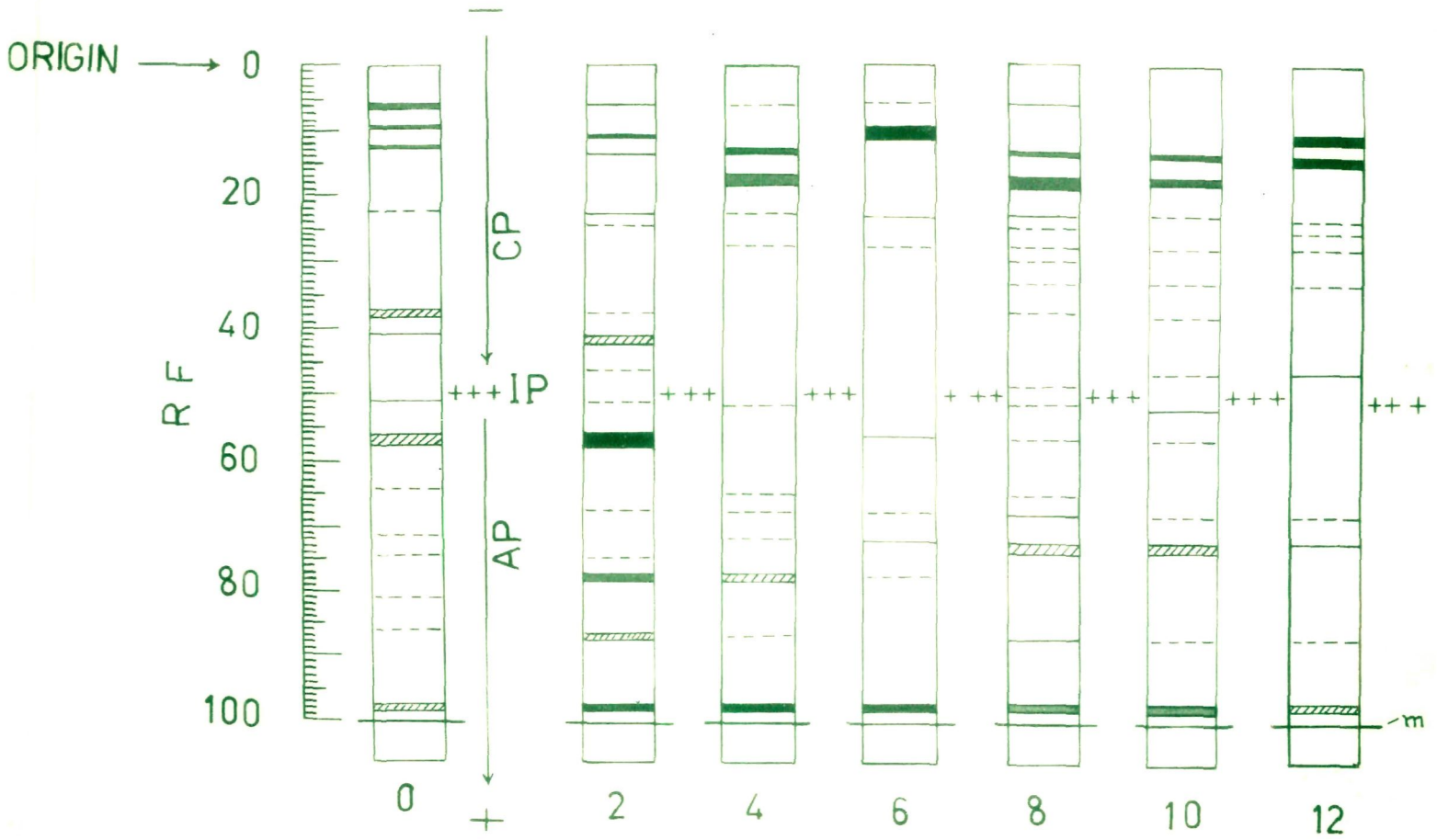
m - marker

▬ - major protein bands

 low protein bands

——— - feeble protein bands

Fig. 12



Rf 12 of the 4 day old ovaries whereas the major band Rf 16 newly appeared at this age. The strongest fraction Rf 97 continued from the previous age. It was interesting to record that the new major fractions, Rf's 56 and 77 of the 2 day old ovaries considerably reduced and only Rf 77 persisted as low fraction in the 4 day old ovaries. However, there were eight feeble fractions, Rf's 6, 22, 27, 51, 64, 67, 71 and 86 among which Rf's 6, 22 and 86 previously appeared as low fractions while Rf's 51 and 67 continued as feeble fractions from the previous age. The fractions, Rf's 24, 37, 46 and 74 (feeble) disappeared. The feeble fractions, Rf's 64 and 71 of the ovaries of the newly emerged affected females, reappeared in the 4 day old ovaries whereas fraction Rf 27 was detected for the first time as a new feeble fraction. Thus as compared to the normal ovarian pattern of the 4 day old females, one major (Rf 56) and two low (Rf's 41 and 74) protein bands disappeared in the ovaries of the affected females of the corresponding age, reducing the total number of the protein bands. Further, the major bands of the ovaries of the affected females were qualitatively weaker in concentration than that of the normal ovarian proteins.

In the ovaries of the 6 day old affected females there was further reduction in the protein fractions and the total number was 9 as compared to 10 in the normal ovaries of this age. There were only two strong major bands, Rf's 9 and 97 in

the ovaries of the 6 day old affected females in which the latter band persisted from the previous age. The less prominent major bands, Rf's 12 and 16 of the 4 day old ovaries apparently fused during migration forming the most voluminous major fraction Rf 9 of the 6 day old ovaries. However, there were three low fractions, Rf's 22, 56 and 71 and among these Rf's 22 and 71 transformed from the respective feeble bands of 4 day old ovaries while band Rf 56 reappeared from the 2 day old females. Among the feeble fractions, Rf's 6, 27 and 67 were retained from the previous age whereas Rf 77 reduced to feeble fraction from the low fraction of the ovaries of the 4 day old females. Thus, the removal of the three feeble fractions, Rf's 51, 64, and 81 from the 4 day old ovaries caused reduction in the total protein fractions of the 6 day old ovaries.

At the age coinciding the first post oviposition period i.e. 8 day following emergence, numerous feeble fractions appeared raising the total number of the protein fractions to a maximum extent i.e. 17 (Fig. 15). There were three major protein bands, Rf's 12, 16 and 97 like the normal ovaries of the 8 day old females. But these bands were less prominent than those of the normal females. Similar pattern was seen in the 4 day old ovaries. The voluminous band Rf 9 of the 6 day old ovaries seemed to be reduced in concentration and broke into less prominent major fractions, Rf's 12 and 16 of the 8 day old ovaries. The major fraction Rf 97 continued

Fig. 13. Number of electrophoretically separated protein bands of the ovaries of normal as well as affected females of *C. cinctipes* related with age.

-o- = Normal ovary

-●- = Affected with Lindane

-▲- = Affected with Parathion

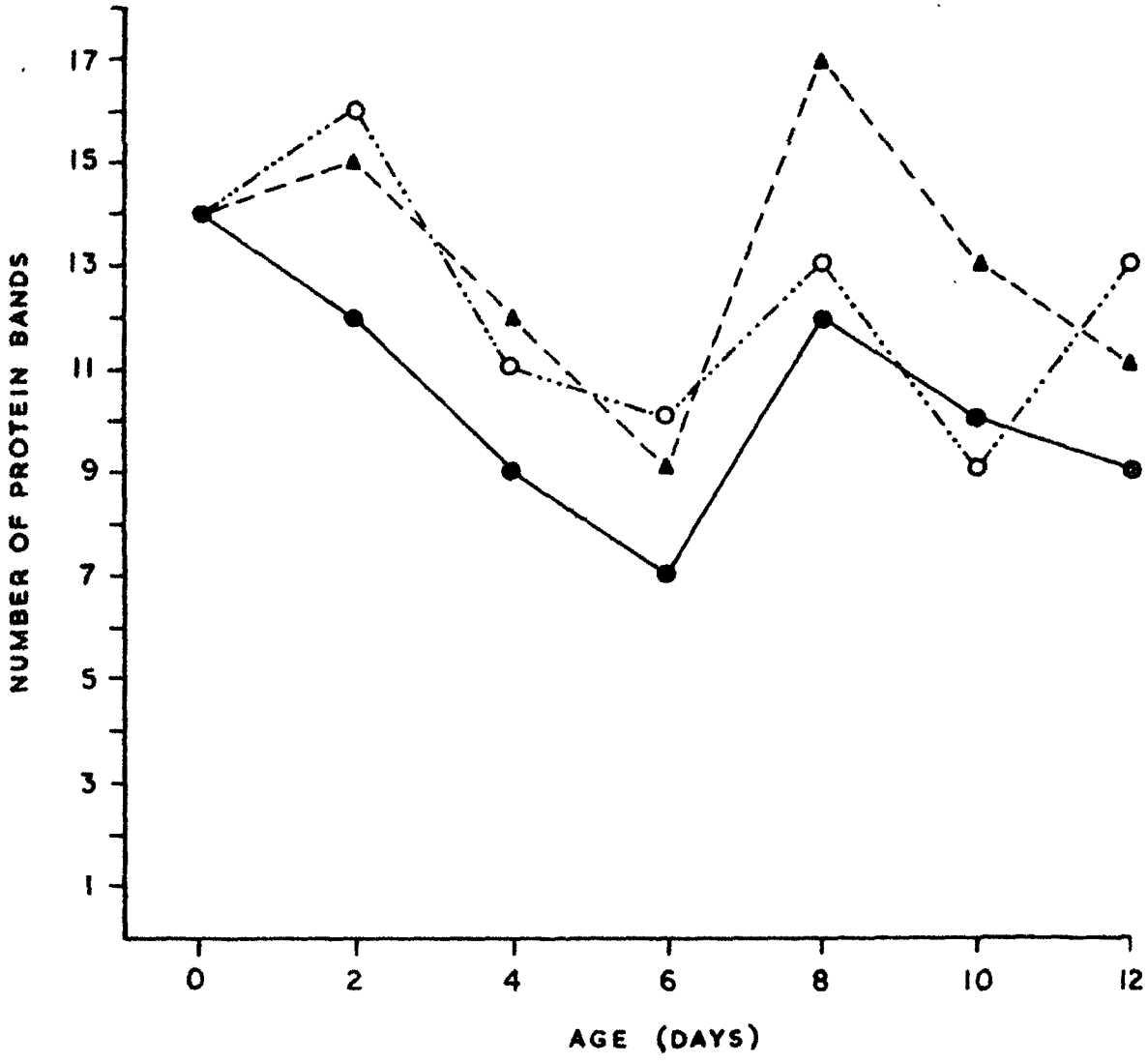


FIG. 13

unchanged. Among the minor bands, there were five low bands, Rf's 6, 22, 67, 71 and 86; nine feeble bands, Rf's 24, 27, 29, 32, 37, 48, 51, 56 and 64. The low Rf 56 and feeble Rf's 6 and 67 of the 6 day old ovaries became feeble and low fractions respectively in 8 day old ovaries. The fractions continued from the 6 day old ovaries were Rf's 22 and 71 (low)and Rf27 (feeble). The feeble fraction Rf 77 of the 6 day old ovaries disappeared. The other minor fractions, Rf 86 (low) and Rf's 24, 37, 51 and 64 (feeble) reappeared together with some new feeble fractions, Rf's 29, 32 and 48 raising the total number of the protein fractions in the ovaries of the 8 day old affected females.

Later, there was again decrease in the number of the protein fractions which was limited to 13 in the ovaries of the ten day old affected females (Fig. 13). The three major fractions, Rf's 12, 16 and 97 continued from the younger ovaries and these were qualitatively higher in concentration than before. However, there was only two low fractions, Rf's 51 and 71 which had appeared as feeble and low fractions respectively in the previous age. The low fractions, Rf's 22, 67 and 86 of the 8 day old ovaries decreased in concentration and continued as feeble fractions in the ten day old ovaries. The feeble fractions, Rf's 27, 32, 37 and 56 persisted from the previous age whereas band Rf 46 of the 2 day old ovaries reappeared at this age. The minor fractions of the 8 day old ovaries lost during the development within two days were Rf 6 (low) and

Rf's 24, 29, 48 and 64 (feeble) decreasing the total number of the protein fractions in ten day old ovaries. The electrophoretic pattern of the separable protein fractions in the ten day old ovaries qualitatively showed lighter concentration in the major bands, reduction in the low bands and increase in the feeble bands indicating weaker concentration of the proteins as compared to that of the normal protein pattern of the ten day old females.

There were only 11 protein fractions in the ovaries of the 12 day old affected females than in that of the normal ovaries of the corresponding age (Table 12 & 18). There were only two major fractions, Rf's 9 and 12. The fraction 12 continued from the previous age while band Rf 9 of the 6 day old ovaries reappeared at this age but it was not as voluminous as before. The most prominent band Rf 97 of the previous age considerably decreased and turned into a low fraction of this age. The other two low fractions were Rf's 46 and 71 which continued from the feeble and the low fractions respectively of the previous age. The feeble fractions, Rf's 22, 27, 32, 67 and 86 continued unchanged whereas fraction Rf 24 of the 8 day old ovaries reappeared at this age. The minor fractions disappeared from this age were Rf's 51 (low), 37 and 56 (feeble) of the previous age. Thus, the presence of the strong major bands, addition of the low bands and removal of some feeble fractions comparatively suggested an increase in the protein concentration of the separable fractions as compared to that

Table - 19

Difference between electrically charged protein fractions in the ovaries of D. cingulatus of different age treated with Parathion (nymphal treatment).

Age (days)	No. of cathodic proteins (-)	No. of anodic proteins (+)	Total number of protein bands
Zero	6	8	14
2	8	7	15
4	5	7	12
6	4	5	9
8	10	7	17
10	7	6	13
12	7	4	11

of the previous age.

In the immature ovaries of the newly emerged females affected with parathion , the number of negatively charged (cathodic) as well as positively charged (anodic) proteins were similar to that of the ovarian pattern of the normal females of the corresponding age (Tables 13 & 19) but varied with the advancing age of the affected females. In the ovaries of the 2 day old affected females, the cathodic proteins increased to 8 and later declined considerably up to 6 day following emergence reaching to a minimum level of 4 bands at the time coinciding the fully matured condition of the normal ovaries. Afterwards, within a couple of days, these negatively charged proteins again increased to a maximal level of 10 bands. Again it was followed by fall which was maintained up to 12 day following the emergence unlike the normal ovarian pattern.

On the other hand, the anodic proteins started decreasing from the beginning but the reduction was slow as compared to that of the normal pattern with respect to age (Table 13 and 19). The decline in the anodic proteins of the affected ovaries was more pronounced in the second half as compared to that of the first half of the total duration (Table 19). The range of variation in the cathodic proteins was 4 to 10 whereas it was 5 to 8 in anodic proteins.

Fig. 14. Histogram showing comparative number of electrically charged protein bands in the ovaries of normal as well as affected *C. circulatus* (adult) of different age.

 - Cathodic proteins

 - Anodic proteins

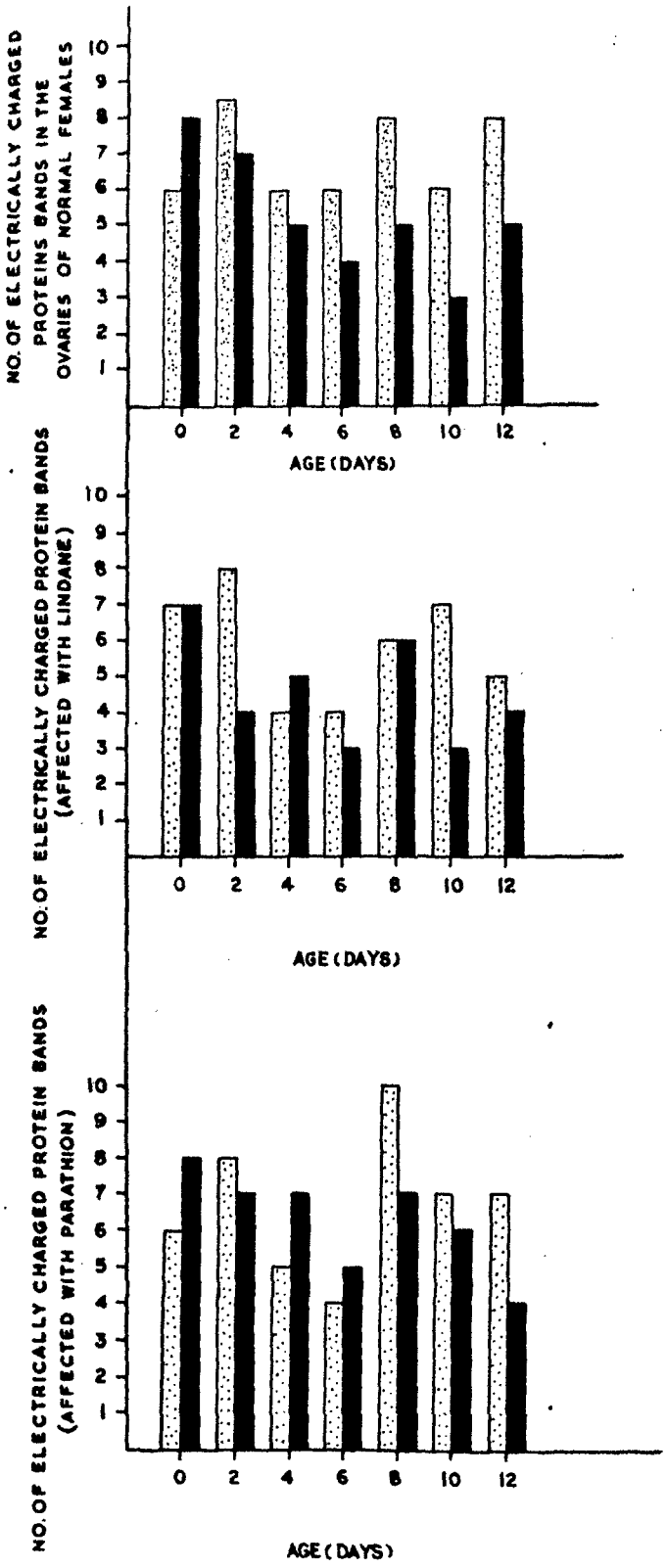

NO. OF ELECTRICALLY CHARGED PROTEINS BANDS IN THE OVARIES OF NORMAL FEMALES
NO. OF ELECTRICALLY CHARGED PROTEIN BANDS (AFFECTED WITH LINDANE)
NO. OF ELECTRICALLY CHARGED PROTEIN BANDS (AFFECTED WITH PARATHION)
AGE (DAYS)
0 2 4 6 8 10 12
1 2 3 4 5 6 7 8 9 10

FIG. 14

Thus, as compared to the normal pattern of the charged proteins, it was observed that within six days of ovarian development in affected ovaries, the trend of variation in both types of charged proteins was same but the reduction in the cathodic proteins was higher than that of the anodic proteins within this period i.e. just reverse to that of the normal pattern. Later, in the second half of development of the affected ovaries, the pattern completely changed from that of the normal one. Thus, in ten days following emergence, there was more reduction in the negatively charged proteins than that of the positively charged fractions. Further, unlike normal ovaries, there was no increase in the cathodic as well as the anodic proteins in the ovaries of the 12 day old affected females (Table 13 and 19). On comparing with the variation range of the normal pattern, it was found that the range of variation in the negatively charged proteins increased while variation range of the positively charged proteins becoms shorter in the ovaries of the affected females. It was also observed that variation range of the cathodic proteins was more affected with the treatment of this insecticide (Fig. 14).

XXI VARIATIONS IN THE TOTAL REDUCING SUGARS IN THE WHOLE BODY OF THE 4th AND THE 5th INSTAR NYMPHS OF *D. CINGULATUS* IN RELATION TO AGE, MOULTING AND METAMORPHOSIS.

The present study deals with the estimation of the total reducing sugars (TRS) in the whole body as per mg body weight of the nympho (4th and 5th instar) of different age from the day of moulting to the metamorphosis in *D. cingulatus*. The average value of the TRS concentration was calculated out of ten individuals of each age in both nymphal instars.

During the 6 days period of the 4th instar nympho of various age, the range of the reducing sugars level of the body was 0.00101 ± 0.00006 mg/mg body weight to 0.00193 ± 0.0001 mg/mg body weight. The concentration of the reducing sugars in the newly moulted 4th instar nymphs varied between 0.00109 mg/mg to 0.00233 mg/mg (Appendix table 7) with a mean value 0.00167 ± 0.0001 mg/mg body weight. Later, this level of the total reducing sugars increased with the advancement of age upto 4 days and the initial enhancement was much rapid (Fig. 15, Table 20). However, the mean value of the reducing sugars level in the 3 day old nymphs was the only statistically significant rise which showed variation between 0.00154 mg/mg to 0.00260 mg/mg (mean value 0.00193 ± 0.0001 mg/mg). It was also the maximal concentration during the 4th instar. Thereafter, the level of the reducing sugars started declining as the nymphs

Table - 20

Variations in total reducing sugars in the whole body of the 4th instar nymphs of D. circulatus in relation to age and moulting.

Age (days)	Total reducing sugars/mg of body weight (mg/mg)	S.E.	't' value
Zero	0.00167	± 0.0001	Control
1	0.00186	± 0.00003	1.032
			0.282
2	0.00190	± 0.0001	1.732
			0.550
3	0.00193	± 0.0001	2.121*
			1.414
4	0.00173	± 0.0001	0.620
			0.500
5	0.00167	± 0.0005	0.500
			3.464*
6	0.00101	± 0.00006	4.242*
			0.845
Moulted 5th instar	0.00091	± 0.00027	

(Table value of 't' at 5% level 18 = 2.101)

Fig. 15. Changes in the total reducing sugars level per mg body weight of the 4th and the 5th instar nymphs of D. cingulatus in relation to age, moulting and metamorphosis.

-o- - reducing sugars level/mg

-●- - body weight of the nymphs

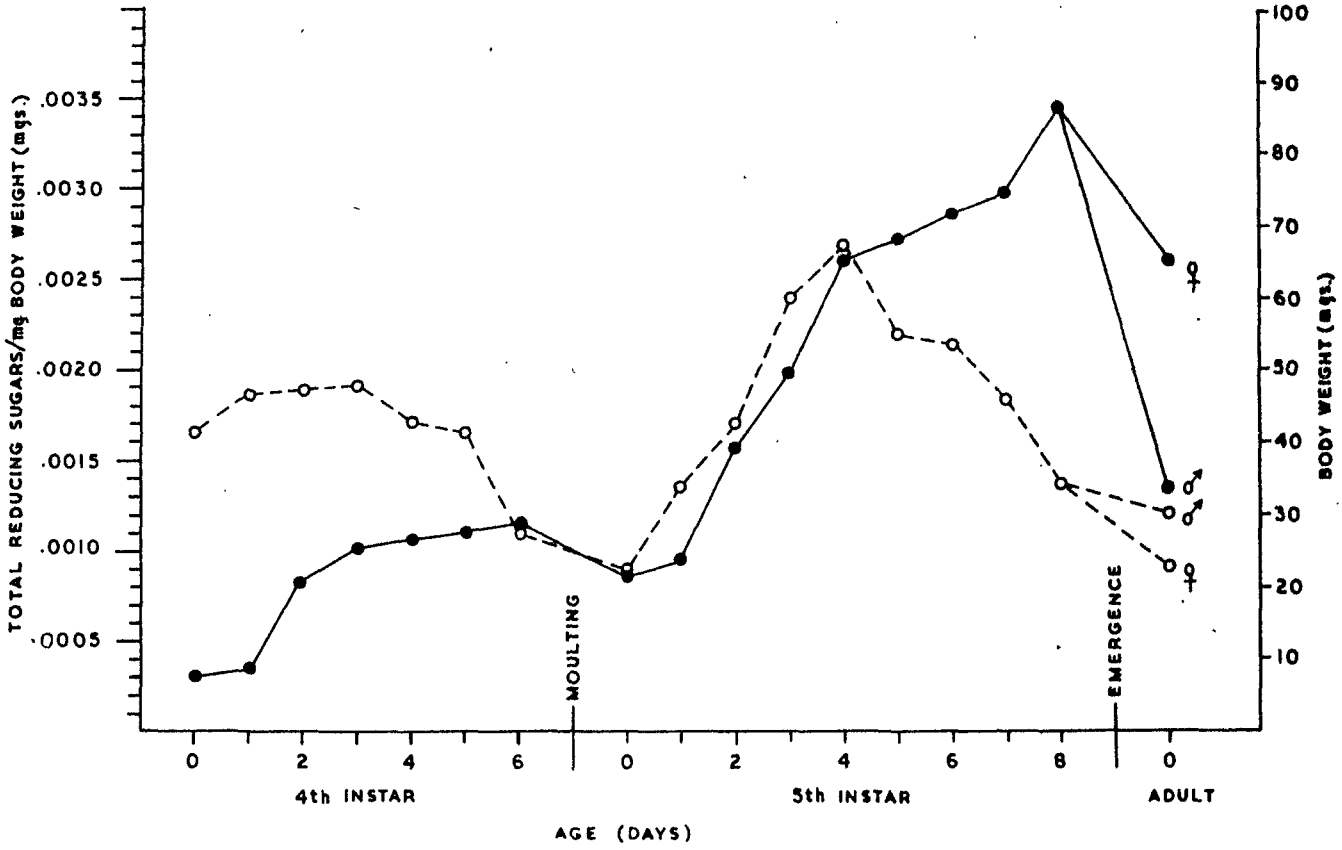

TOTAL REDUCING SUGARS/mg BODY WEIGHT (mgs.)
.0035
.0030
.0025
.0020
.0015
.0010
.0005
BODY WEIGHT (mgs.)
100
90
80
70
60
50
40
30
20
10
MOULTING
EMERGENCE
0
2
4
6
0
2
4
6
8
0
4th INSTAR
5th INSTAR
ADULT
AGE (DAYS)
♀
♂
♂
♀

FIG. 15

became older (Table 20 and Fig. 15). But the TRS level was still higher in the 4 day old nymphs than that of the newly moulted nymphs. The fall in the TRS level was statistically insignificant as compared to the maximal concentration. However, in the 6 day old nymphs of this instar, the reduction in the TRS level was remarkable and statistically significant. The mean value was 0.00101 ± 0.00006 mg/mg body weight which was also significantly lower than that of the newly moulted nymphs of the 4th instar (Table 20). Therefore, the fully grown 4th instar nymphs had lost a significant amount of the TRS by the time they attained maximum weight of the body before the next moulting to the 5th instar. Thus, during the 4th instar, the nymphs gained about 0.00026 mg reducing sugars and subsequently 0.00092 mg of the TRS was lost in the second half of the instar.

The newly moulted nymphs to the 5th instar had variation in the TRS level of the body between 0.00066 mg/mg to 0.00102 mg/mg (Appendix Table 7) and its mean value was 0.00091 ± 0.00027 mg/mg body weight. This level was lower, although statistically insignificant as compared to that of the nymphs of the 4th instar just before moulting to the 5th instar (Table 20).

However, the concentration of the TRS in the newly moulted 5th instar nymphs (zero day) was almost half of the value in one day old 4th instar nymphs (Table 20). Like the 4th instar nymphs, those of the 5th instar also gained in the

Table - 21

Variations in total reducing sugars in the whole body of the 5th instar nymphs of R. circulatus in relation to age and final moulting.

Age (days)	Total reducing sugars/mg of body weight (mg/mg)	S.E.	't' value	
Zero	0.00091	± 0.00027	Control	
1	0.00137	± 0.00018	1.928	
				1.632
2	0.00170	± 0.00016	4.276*	
				2.745*
3	0.00241	± 0.00019	7.071*	
				1.133
4	0.00270	± 0.00018	8.889*	
				2.024
5	0.00221	± 0.00016	6.948*	
				0.408
6	0.00215	± 0.00020	5.515*	
				1.224
7	0.00184	± 0.00015	5.031*	
				2.041
8	0.00137	± 0.00019	1.865	
			1.845	
Emerged female	0.0009	± 0.00029		
			0.435	
Emerged male	0.0012	± 0.00012		

(Table value of 't' at 5% level 18 = 2.101)

concentration of the reducing sugars within 24 hours following moulting but it was statistically insignificant (Table 21). The range of variation in the TRS level of the body of one day old 5th instar nymphs was between 0.00088 mg/mg to 0.000180 mg/mg and the mean value was 0.00137 ± 0.00018 mg/mg body weight. However, within a couple of days the TRS level significantly increased to the value which was nearly double to that of the newly moulted 5th instar nymphs (Table 21). The trend of the enhancement in the TRS level continued up to 4 days following moulting and it was statistically significant in 2, 3 and 4 day old nymphs contrary to the changes in the 4th instar nymphs. Further, after two days following moulting the increase in the TRS concentration was faster than the initial changes (Table 21). Thus, in the 4 day old 5th instar nymphs, the TRS level reached to almost three times (0.00270 ± 0.00018 mg/mg body weight) to that of the concentration (0.00091 ± 0.00027 mg/mg body weight) in the newly moulted nymphs.

Later, like the 4th instar nymphs, there was statistically significant fall in the level of the TRS of the body in the five day old nymphs of this instar (t = 2.024). The range of variation in the TRS among the 5 day old nymphs was between 0.00167 mg/mg to 0.00278 mg/mg and average value was 0.00221 ± 0.00016 mg/mg body weight. The decreasing trend in the TRS further continued but it was statistically insignificant between 6 and 7 day old nymphs and the mean values were 0.00215 ± 0.00020 mg/mg and 0.00184 ± 0.00015 mg/mg body weight respectively.

However, the significant loss in the TRS occurred from the previous age in the fully grown nymphs i.e. the 8 day old 5th instar nymphs prior to the metamorphosis to the adult stage (t = 2.041). The reduced level of the TRS in these nymphs varied between 0.00097 mg/mg to 0.00183 mg/mg having a mean value 0.00137 ± 0.00019 mg/mg body weight which was similar to the level found in the one day old nymphs of this stage (Table 21).

Thus, the quantity of the reducing sugars increased within the first four days of the 5th instar and it was 0.00179 mg. Whereas the amount lost in the later half was 0.00133 mg. Therefore, unlike the 4th instar nymphs, higher amount of the TRS content was introduced in the first half of the 5th instar and a lesser amount of the TRS was utilised in the remaining half period before the metamorphosis to the adult. The increase in the TRS level throughout the whole duration of the 5th instar nymphs occurred by the addition of 0.00046 mg of the TRS content. The range of variation in the TRS concentration during the 8 day period of the 5th instar remained 0.00091 ± 0.00027 mg/mg body weight to 0.00270 ± 0.00018 mg/mg body weight. However, the fully grown nymphs of this instar had an increase of 0.00046 mg in the TRS level over that of the youngest nymphs of this stage (newly moulted). Further, it is also clear that the fully grown nymphs before metamorphosis had higher level of the reducing sugars of the body than that of the fully grown nymphs of the previous stage (Fig. 15). It

was interesting to record that during both the instar periods the course of increase in the ZNS level and the body weight was almost parallel in the first half whereas it was just reversed in the second half of the respective instars (Fig. 15).

Generally, after 8 days following the moulting to the 5th instar, the nymphs metamorphosed into the adults and the ZNS level in the newly emerged adults both the males and the females was remarkably lower than that of the fully grown nymphs of the last stage but the values were statistically insignificant (Table 21). Further, the newly emerged females had less ZNS concentration than that of the males of the corresponding stage.

XIII. VARIATIONS IN THE TOTAL REDUCING SUGARS IN THE WHOLE BODY OF ADULT *D. CINGULATUS* RELATED WITH AGE AND REPRODUCTION.

Variations were observed in the total reducing sugars (TRS) level of each sex during adult development and reproduction within 12 days from emergence.

In the newly emerged males the TRS varied between 0.00062 mg/mg to 0.00168 mg/mg averaging 0.001204 ± 0.00012 mg/mg body weight. Initially the synthesis of the reducing sugars in the body was slow. However, the TRS level of the body was maximum in the 3 day old males, 0.001413 ± 0.000137 mg/mg but statistically insignificant (Table 22). The range of variation among the different males of this age was between 0.00085 mg/mg to 0.00177 mg/mg (Appendix table 8). It was followed by a sharp fall to the level of 0.001186 ± 0.000176 mg/mg body weight. However, this decrease was statistically insignificant when compared with the concentration of the 3 day old males ($t = 1.341$). Subsequently, the fall continued further in the older males up to 9 days following emergence. The most remarkable and statistically significant decrease was observed in the males aged 8 and 9 days (Table 22) which had TRS concentration 0.000882 ± 0.000141 mg/mg body weight and 0.000723 ± 0.000332 mg/mg body weight respectively. However, there was again rise in the TRS which progressively reached to 0.001221 ± 0.000163 mg/mg body weight within a couple of days (Fig. 16).

Table - 22

Variation in total reducing sugars in the whole body of adult *D. cingulatus* related with age and reproduction

Age (days)	MALE: Total reducing sugars in male/mg body weight (mg/mg) S.E.	't' value	FEMALE: Total reducing sugars in female/mg body weight (mg/mg) S.E.	't' value
Zero	0.001204 ± 0.000120	Control	0.000936 ± 0.000297	Control
1	0.001102 ± 0.000148	0.790	0.001092 ± 0.000148	1.906
2	0.001315 ± 0.000153	1.154 / 0.559	0.000915 ± 0.000239	1.348 / 0.250
3	0.001413 ± 0.000157	0.577 / 1.100	0.001018 ± 0.000258	0.223 / 0.816
4	0.001186 ± 0.000176	1.341 / 0.471	0.001335 ± 0.000124	0.866 / 2.760*
5	0.000958 ± 0.000133	0.912 / 1.677	0.001304 ± 0.000110	0.670 / 2.981*
6	0.000964 ± 0.000120	1.677 / 1.792	0.001415 ± 0.000278	0.707 / 3.446*
7	0.000938 ± 0.000105	0.894 / 1.897	0.000952 ± 0.000141	3.042* / 0.766
8	0.000882 ± 0.000141	0.577 / 2.169*	0.000938 ± 0.000297	3.952* / 0.117
9	0.000725 ± 0.000332	0.577 / 3.162*	0.000982 ± 0.000141	0.111 / 0.235
10	0.001014 ± 0.000297	2.121* / 1.318	0.001197 ± 0.000140	1.000 / 1.135
11	0.001221 ± 0.000163	2.886* / 0.197	0.000882 ± 0.000133	1.500 / 0.620
12	0.001015 ± 0.000081	1.380	0.000706 ± 0.000137	2.454* / 1.217

(Table value of 't' at 5% level 18 = 2.101)

Although this rise in the TRS of the body of the 11 day old males was statistically insignificant as compared to that of the control level, it was significantly higher than that of the TRS level of the 9 day old males (Table 22). However, in the 12 day old males the TRS concentration again dropped to the level of 0.00103 ± 0.0000815 ng/ng body weight. Therefore, the range of variation in the TRS level during the twelve day old life of the males was between 0.000723 ± 0.0000332 ng/ng body weight to 0.001413 ± 0.000137 ng/ng body weight.

As mentioned before, in the newly emerged females (zero day), the TRS concentration of the body was insignificantly lower than that of the males of the corresponding age and ranged between 0.00069 ng/ng to 0.00133 ng/ng with a mean value 0.000936 ± 0.000297 ng/ng body weight. Thereafter, the TRS level insignificantly enhanced within 24 hours (0.001092 ± 0.000148 ng/ng body weight). However, it was not followed by further increase within the next 48 hours following emergence. But the TRS level of the 4 day old females was significantly higher than before, i.e. 0.001335 ± 0.000124 ng/ng body weight which was almost equal to the concentration in the fully grown nymphs before metamorphosis (Table 21). However, the TRS level remained significantly high in the maturing females of 4 to 6 day old and the increase was more pronounced when the females fully matured and they were ready to deposit the first batch of eggs (Table 22). Later, following the oviposition on the 7th and the 8th post-emergent days, the level of

the TRS dropped and it was significant change in the 8 day old females as compared to that of the 6 day old females (t = 3.952). Following the oviposition, there was further rise in the TRS level of the body as the female matured second time and the second peak of the TRS concentration (0.001197 ± 0.000140 mg/mg body weight) was on the 10th post emergent day, which was statistically insignificant unlike the first peak (Table 22). Also, the difference between the two peaks was insignificant (t = 1.642). Later, there was progressive decline in the contents of the TRS reaching a minimum value of 0.000706 ± 0.000137 mg/mg body weight in the 12 day old females. This fall was insignificant as compared to the TRS level of the control but it was significant with respect to that of the second peak of the TRS concentration (t = 2.454). Another interesting point noticed was that following the first oviposition, the level of the TRS of the body was almost equal to that of the newly emerged females whereas following the second oviposition the TRS level dropped to a concentration much below that of the control i.e. newly emerged females (Fig. 16).

As compared to the newly emerged females, the TRS level was insignificantly higher in the males of the corresponding age and maximum concentration was recorded (0.001413 ± 0.000137 mg/mg body weight) in the 3 day old males whereas almost similar level of the TRS (0.001413 ± 0.000278 mg/mg body weight) was synthesised in the body of the 6 day old females. In the males, there was continuous decline in the TRS level following

Fig. 16. Changes in the total reducing sugars level per mg body weight of the adult *D. simulatus* related with age.

-●- = total reducing sugars level in the whole body of male/mg

-o- = total reducing sugars level in the whole body of female/mg

..Δ.. = body weight of the female

...▲... = body weight of the male

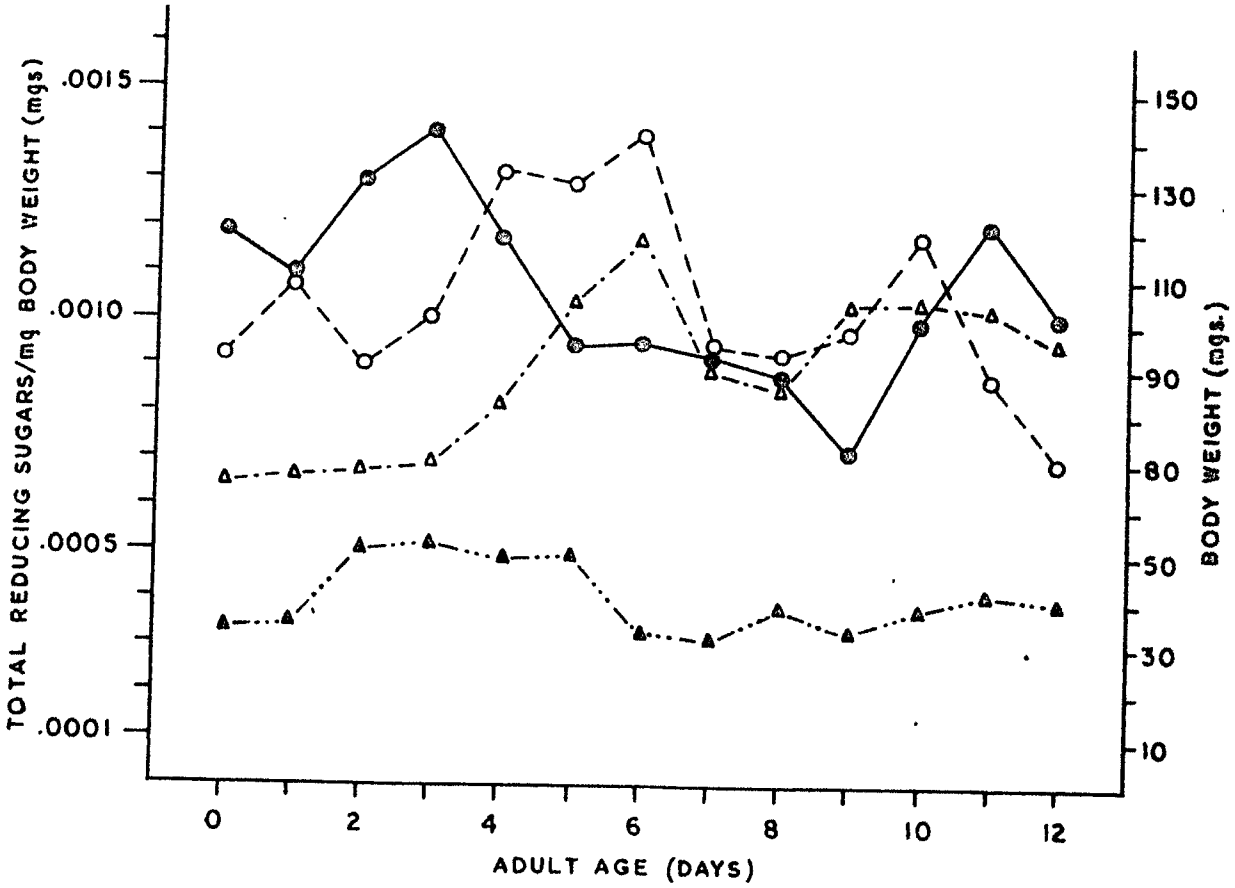

TOTAL REDUCING SUGARS/mg BODY WEIGHT (mgs)
.0015
.0010
.0005
.0001
BODY WEIGHT (mgs.)
150
130
110
90
80
50
30
10
0
2
4
6
8
10
12
ADULT AGE (DAYS)

FIG. 16

emergence and significantly low level was found in the 8 and the 9 day old males. However, there was again significant rise in the 11 day old males. As compared to this situation, in the females, the peaks of the TRS level in the body were related with the preoviposition periods (Fig. 16).

There was statistically insignificant difference in the TRS level of the two sexes at the time of emergence ($t = 1.978$). However, the TRS level of the two sexes statistically differed within the first 6 days following emergence especially among the sexes belonging to 2, 3, 5 and 6 days ($t = 2.637$, 2.529, 2.309 and 3.370 respectively). In the last age group, the difference was highest ($t = 3.370$). During the rest of the period the difference was insignificant.

Further, during the 12 days period, the trend of variation in the concentration of the TRS in the whole body of the males is different as compared to that of the total body weight. Whereas in the females, the changes in the TRS level are parallel to that of the body weight (Fig. 16).

XIV. VARIATIONS IN THE TOTAL REDUCING SUGARS LEVEL IN THE OVARIES OF *D. CIRCULATUS* RELATED WITH AGE AND REPRODUCTION.

The most conspicuous changes in the TRS level of the whole body of the females related with the reproductive cycles, suggested to investigate the changes in the TRS level in the ovaries of individual female of different age during the 12 post-emergent days. For this purpose, the TRS was determined per mg of the ovaries. In the ovaries of the newly emerged females, there was small amount of the TRS ranging from 0.0040 mg/mg to 0.00102 mg/mg with a mean value 0.000696 ± 0.0001333 mg/mg ovarian tissue (OT). Later, for a couple of days it did not increase and remained almost the same (Table 23). However, in the 3 day old females, ovarian reducing sugars (ORS) level enhanced (0.000997 ± 0.000159 mg/mg OT). The most striking and significant increase in the ORS level occurred in the 5 and the 6 day old females, having the mean values 0.001017 ± 0.000256 mg/mg OT and 0.00121 ± 0.000278 mg/mg OT respectively. Thus, significantly highest concentration of the ORS was found in the 6 day old females (Table 23 and Fig. 17). Following the oviposition of the first batch of the eggs in between 7th and 8th post-emergent days, the ORS level significantly decreased to 0.000774 ± 0.00166 mg/mg tissue weight in the 7 day old females ($t = 2.672$). This value was still higher although insignificantly as compared to the ovaries of the newly emerged females (Table 23).

Table - 23

Variation in total reducing sugars in the ovaries of D. circulatus in relation to age and reproduction

Age (days)	Total reducing sugars in the ovaries/mg tissue weight (mg/mg) S.E.	't' value
Zero	0.000686 ± 0.000133	Control
1	0.000657 ± 0.000120	0.111
		0.462
2	0.000659 ± 0.000166	0.942
		1.341
3	0.000997 ± 0.000159	1.446
		0.577
4	0.000825 ± 0.000110	1.154
		1.533
5	0.001017 ± 0.000258	2.581*
		1.690
6	0.001212 ± 0.000278	3.794*
		2.672*
7	0.000774 ± 0.000166	0.471
		1.039
8	0.000923 ± 0.000316	1.825
		0.707
9	0.001014 ± 0.000110	2.434*
		0.645
10	0.001126 ± 0.000120	2.795*
		2.480*
11	0.000707 ± 0.000110	0.577
		3.872*
12	0.000521 ± 0.000316	0.608

(Table value of 't' at 5% level 18 = 2.101)

Fig. 17. Changes in the total reducing sugars level per mg of ovaries of the adult _. circulatus related with age.

·o· - total reducing sugars level in the ovary/mg

·●· - total reducing sugars level in the whole body of female/mg

·▲· - weight of the ovary

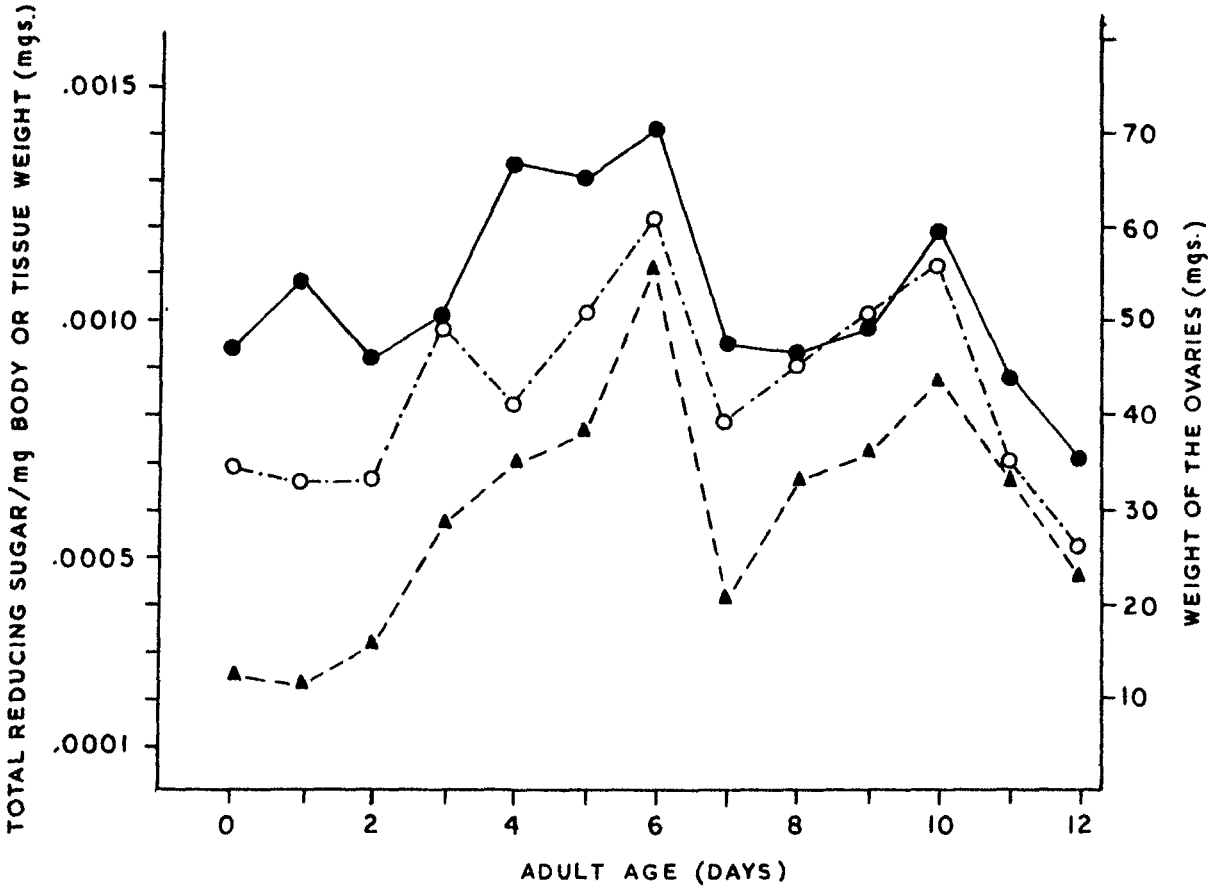

TOTAL REDUCING SUGAR/mg BODY OR TISSUE WEIGHT (mgs.)
.0015
.0010
.0005
.0001
0
2
4
6
8
10
12
ADULT AGE (DAYS)
70
60
50
40
30
20
10
WEIGHT OF THE OVARIES (mgs.)

FIG. 17

Afterwards, as the ovaries further matured more rapidly than before, there was progressive synthesis of the ORS up to 10 days following emergence. Thus, in the ovaries of 8, 9 and 10 day old females, the mean values of the ORS level was 0.000923 ± 0.000316 mg/mg, 0.001014 ± 0.000110 mg/mg and 0.001126 ± 0.000120 mg/mg tissue weight respectively. Thus, a second peak of the ORS level occurred in the 10 day old females (Table 23 and Fig. 17). Although, the first peak was arithmatically higher than that of the second, the difference was statistically insignificant. Again, during the post-oviposition of the second batch of the eggs i.e. in the ovaries of the 11 and the 12 day old females, the ORS level again significantly dropped to 0.000707 ± 0.000110 mg/mg OT (t = 2.480) and 0.000521 ± 0.000316 mg/mg OT (t = 3.672) respectively with regard to the peak concentration of the ORS in the 10 day old females. This change in the ORS decline was more significant (t = 3.672) than that following the first oviposition (t = 2.672) with respect to the two peak concentrations. It was interesting to record that following the second oviposition, the ORS level in the ovaries of the 12 day old females was lower (0.000521 ± 0.000316 mg/mg tissue weight) than that of the newly emerged females (0.000686 ± 0.000133 mg/mg tissue weight).

Thus the changes in the ORS level following the first peak concentration are parallel to that of the TRS level of the body (Fig. 17). Similarly the changes in the ovarian weight and the ORS level during the 6 post-emergent days are also parallel.

XV CHANGES IN THE TOTAL FAT CONTENT IN THE 4th AND THE 5th INSTAR NYMPHS OF *D. CINGULATUS* RELATED WITH AGE, MOULTING AND METAMORPHOSIS.

The 4th instar nymphs of *D. cingulatus* just moulted from the 3rd instar nymphs contained maximum concentration of the total fat (174.0 ± 5.549 mg) during this instar and the percentage of total fat was 75.025% with respect to average dry body weight. Later, the total fat concentration (TFC) continued to decrease significantly during the next two days (Table 24 and Fig. 18). Thus, in 1 and 2 day old 4th instar nymphs the TFC was 121.2 ± 9.107 mg and 112.4 ± 2.932 mg of the body weight (dry) respectively. But, consequently, there was almost fifty per cent reduction in the total fat percentage of the body as compared to that of the newly moulted 4th instar nymphs (Fig. 20). Afterwards, there was increase in the TFC of the 3 day old 4th instar nymphs and the mean value reached to 126.4 ± 10.554 mg. On statistical comparison this amount did not appreciably changed in the older nymphs. But the TFC of the nymphs prior to the next moulting was significantly lower than that of the newly moulted 4th instar nymphs, reaching almost half (132.0±19.418 mg) of the maximum TFC (174.0±5.549 mg) of the zero age 4th instar nymphs. However, the dry weight per gm fresh weight of the body gradually dropped to 258.0 ± 13.281 mg in the 6 day old nymphs (fully grown 4th instar nymphs). With respect to the dry body weight,

Table - 24

Changes in total fat content of the 4th instar nymphs of *D. cingulatus* related with age and moulting

Age (days	Dry weight/ one gm fresh weight (mg)	Mean weight of total fat (mg)	't' value	Percentage of total fat to dry weight (%)
Zero	230.0± 5.413	174.0± 5.549	Control	75.826
1	258.2± 7.197	121.2± 9.107	4.950*	46.933
			0.920	
2	292.0± 6.164	112.4± 2.932	9.813*	38.493
			1.278	
3	270.0±11.063	126.4±10.554	3.991*	46.814
			0.193	
4	271.6±13.524	123.6± 9.874	4.449*	45.508
			0.484	
5	262.0±18.609	130.2± 9.162	4.088*	49.694
			0.093	
6	258.0±13.281	132.2±19.418	2.069	51.240
			2.811*	
Moulted 5th instar	250.0±11.247	74.0± 7.169		29.600

(Table value of 't' at 5% level 8 = 2.306).

Fig. 10. Variations in the total fat concentration in the whole body of the developing nymphs (4th and 5th instar) of *P. cingulatus* in relation to age, moulting and metamorphosis.

-●- total fat concentration

-o- dry body weight

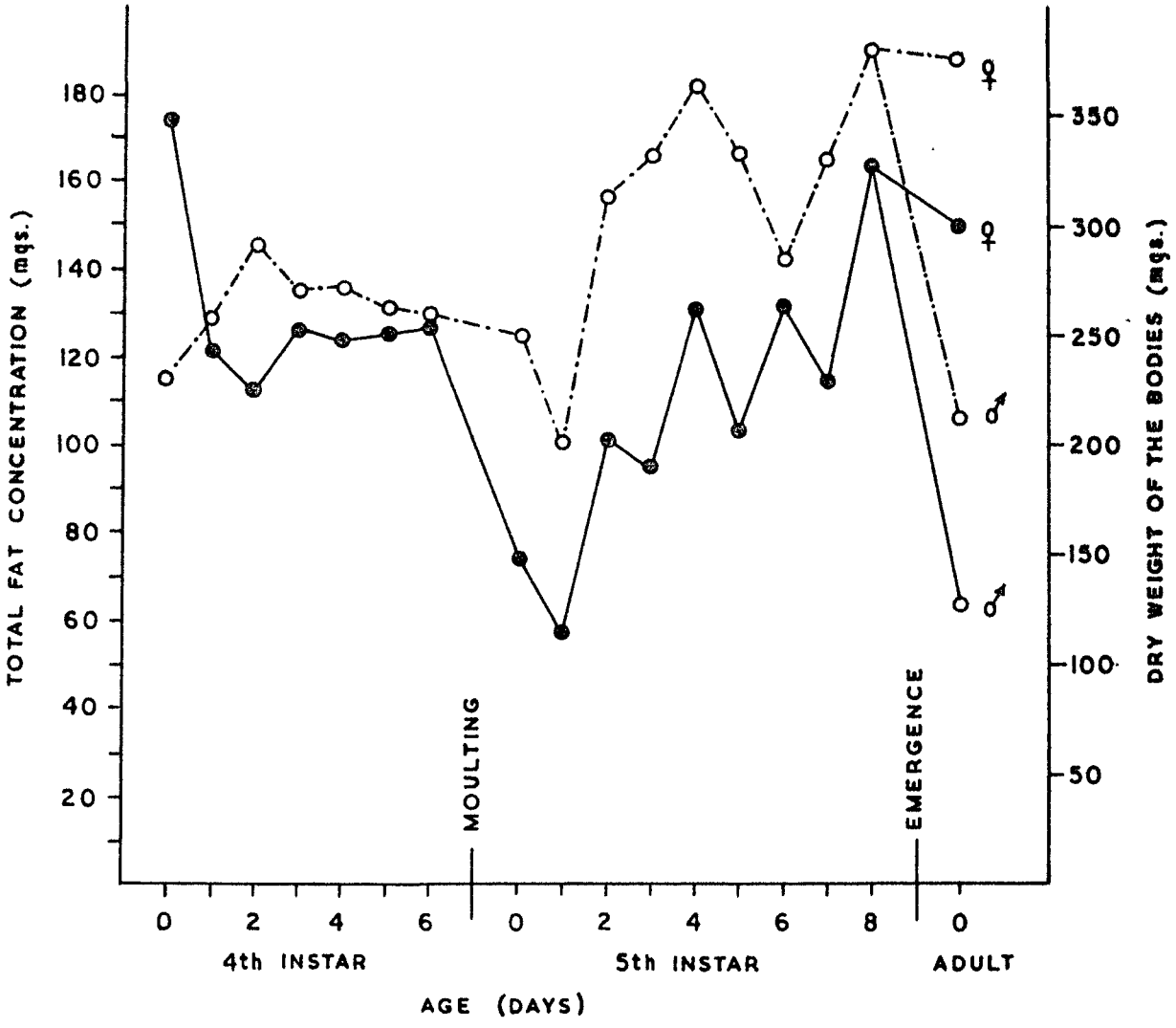

TOTAL FAT CONCENTRATION (mgs.)
DRY WEIGHT OF THE BODIES (mgs.)
180
160
140
120
100
80
60
40
20
350
300
250
200
150
100
50
♀
♀
♂
♂
MOULTING
EMERGENCE
0
2
4
6
0
2
4
6
8
0
4th INSTAR
5th INSTAR
ADULT
AGE (DAYS)

FIG. 18

the percentage of the total fat enhanced to a maximum level of 51.240% in the 6 day old nymphs from the level of 38.493% when the average dry body weight was maximum (Fig. 20).

In the nymphs newly moulted to the 5th instar the TFC significantly dropped and the mean value was 74.0 ± 7.169 mg which was almost half of that of the nymphs ready to moult (Fig. 18). Although the nymphs did not loose much dry body weight the percentage of the total fat was considerably reduced (Fig. 20). Initially, within 24 hours there was no statistically significant change but the TFC in the 2 day old 5th instar nymphs enhanced to 101.4 ± 8.432 mg with the corresponding dry body weight 312.0 ± 10.672 mg. Thus, the TFC in these nymphs was 32.5%. Addition of more fat accompanied with an increase in the dry body weight was recorded in the 4 day old 5th instar nymphs (131.2 ± 11.070 mg). This TFC was almost two fold as compared to that of the newly moulted 5th instar nymphs and as much as that of the 6 day old 4th instar nymphs (Fig. 18).

Following an insignificant fall in the 5 day old nymphs, there was again a significant increase in the percentage of the total fat due to lowering of the dry body weight, but the TFC was to the level of the 4 day old nymphs. Thereafter the TFC fluctuated until the time of metamorphosis. However, in the 8 day old nymphs which were ready to metamorphose both the dry weight and the TFC were highest during this instar (Table 25, Fig. 18), although the percentage of the fat was lower (43.157%) than that of the maximum percentage of this instar (46.043%).

Table - 25

Changes in total fat content of the 5th instar nymphs of *D. cingulatus* related with age and metamorphosis.

Age (days)	Dry weight/ one gm fresh weight (mg)	Mean weight of total fat (mg)	't' value	Percentage of total fat to dry weight (%)
Zero	250.0±11.247	74.0± 7.169	Control	29.6
1	200.0±11.035	57.0± 7.694	1.616	20.5
			3.889*	
2	312.0±10.672	101.4± 8.432	2.457*	32.5
			0.578	
3	330.0±10.686	94.4± 8.666	1.813	28.606
			2.617*	
4	364.0±12.143	131.2±11.070	4.356*	36.043
			1.832	
5	332.0±12.251	103.0±10.689	2.253*	31.024
			1.919	
6	284.5± 7.007	131.0±11.402	4.252*	46.045
			1.170	
7	330.0±11.432	114.2± 8.732	3.558*	34.606
			5.081*	
8	380.0± 9.072	164.0± 4.490	10.666*	43.157
			1.656	
Emerged female	377.0± 8.339	150.0± 7.190		39.787
			10.526*	
Emerged male	212.0±12.442	63.0± 8.505		29.716

(Table value of 't' at 5% level 8 = 2.306).

Thus, the data revealed that the average addition of the total fat, irrespective to the dry weight was 90.0 mg from the beginning to the end of the last instar, 57.2 mg accumulated in the first quarter whereas 32.8 mg was added during the second half of the 5th instar duration (6 days). Generally, the percentage of the fat to the dry weight of the body was comparatively higher in the second half than that of the first half of the 5th instar period, suggesting that a greater amount of the fat was accumulated in the second half of the growing nymphs (Table 25). As compared to the nymphs of the 5th instar, in those of the 4th instar, percentage of the fat was higher due to lighter nymphs (Fig. 20).

XVI CHANGES IN THE TOTAL FAT CONTENT IN ADULT *D. CINGULATUS* IN RELATION TO AGE AND REPRODUCTION.

During the nymphal-adult transformation i.e. metamorphosis, the mean TFC as well as its percentage with respect to dry weight per gramme fresh body weight declined in the newly emerged adults of both sexes (Table 25 & Fig. 20). However, this change was statistically significant in the males at the time of emergence, suggesting that a greater amount of the nymphal fat goes with the emerged females (Table 25).

In the newly emerged females (zero age), the total fat concentration (TFC) of the whole body was more than double (150.0 ± 7.190 mg) as compared to that of the males (63.0 ± 8.503 mg) of the corresponding age and respectively the percentage of the total fat in relation to the dry weight of the body was 39.78% and 29.716%.

In the males within a couple of days following emergence, the mean TFC as well as percentage of the total fat reduced fifty per cent (Table 25). This change was statistically significant. In the older males the TFC was on the decline except small fluctuations during the 12 days period (Fig. 19). Although the mean values of the TFC between the males of the successive age statistically did not differ, that of the 12 day old males was remarkably lowest than that of the newly emerged males (Table 25).

Table - 26

Changes in total fat content of the adults of D. cingulatus related with age and reproduction

Age (days)	MALE: Dry weight/ one gm fresh weight (mg)	MALE: Mean weight of total fat (mg)	MALE: 't' value	MALE: Percentage of total fat to dry weight (%)	FEMALE: Dry weight/ one gm fresh weight (mg)	FEMALE: Mean weight of total fat (mg)	FEMALE: 't' value	FEMALE: Percentage of total fat to dry weight (%)
Zero	212.0±12.442	63.0± 8.503	Control	29.716	377.0± 8.994	150.0± 7.190	Control	39.787
2	277.6± 7.355	35.4± 3.033	3.057*	12.752	355.0±13.450	132.2± 6.477	1.860	37.183
			1.422				2.395*	
4	268.4± 6.519	30.8± 1.118	3.754*	11.475	271.2± 9.442	97.0± 7.668	5.041*	35.766
			1.459				2.006	
6	253.0± 8.497	37.4± 4.381	2.676*	14.782	260.0± 8.803	76.0± 7.119	7.314*	29.230
			0.739				5.459*	
8	240.0± 7.078	31.0± 7.467	2.828*	12.916	233.0±10.531	29.2± 4.776	13.994*	12.532
			0.446				10.345*	
10	237.0± 8.514	27.0± 4.959	3.657*	11.392	251.2±11.711	107.0± 5.762	4.666*	42.595
			1.681				6.829*	
12	217.0± 6.403	16.2± 4.080	4.962*	7.465	238.0± 6.557	34.4± 8.933	10.080	14.453

(Table value at 5% level 8 = 2.306).

It was interesting to observe that about fifty per cent of the TFC was used up within a week from emergence and thereafter only one quarter of the initial concentration was lost during the next 6 days. As compared to this change, initially the dry weight of the body enhanced from emergence and the 2 day old males had 277.4 ± 7.355 mg average dry body weight. Thereafter, this weight steadily decreased to the lowest level in the twelve day old males (Fig. 19).

In the females, the changes in the TFC were more pronounced as compared to that of the males of the corresponding age (Table 26 & Fig. 19). Further, initially, the loss of fat was proportionately slower as compared to that of the males and also it was statistically insignificant. Further onward, as the females grew older and matured, the TFC always remained significantly low during the 12 post-emergent days as compared to that of the newly emerged females (Table 26).

During the first 6 days following emergence, the total fat concentration reduced to approximately half of the quantity of the newly emerged females and its percentage was remarkably low (29.290%). However, the TFC of the 4 day old females was significantly lower than that of the 2 day old females (t = 2.395). But in the 8 day old females, significant loss of the fat reserve occurred from emergence and the quantity was only 29.2 ± 4.776 mg and its percentage was approximately one third of the initial percentage (Table 26 & Fig. 20). It was again followed by a

Fig. 19. Variations in the total fat concentration in the whole body of the adult *D. circulatus* related with age.

...▲...● total fat concentration in the whole body of males

-●- dry body weight of the males

-▲- total fat concentration in the whole body of females

..o.. dry body weight of the females

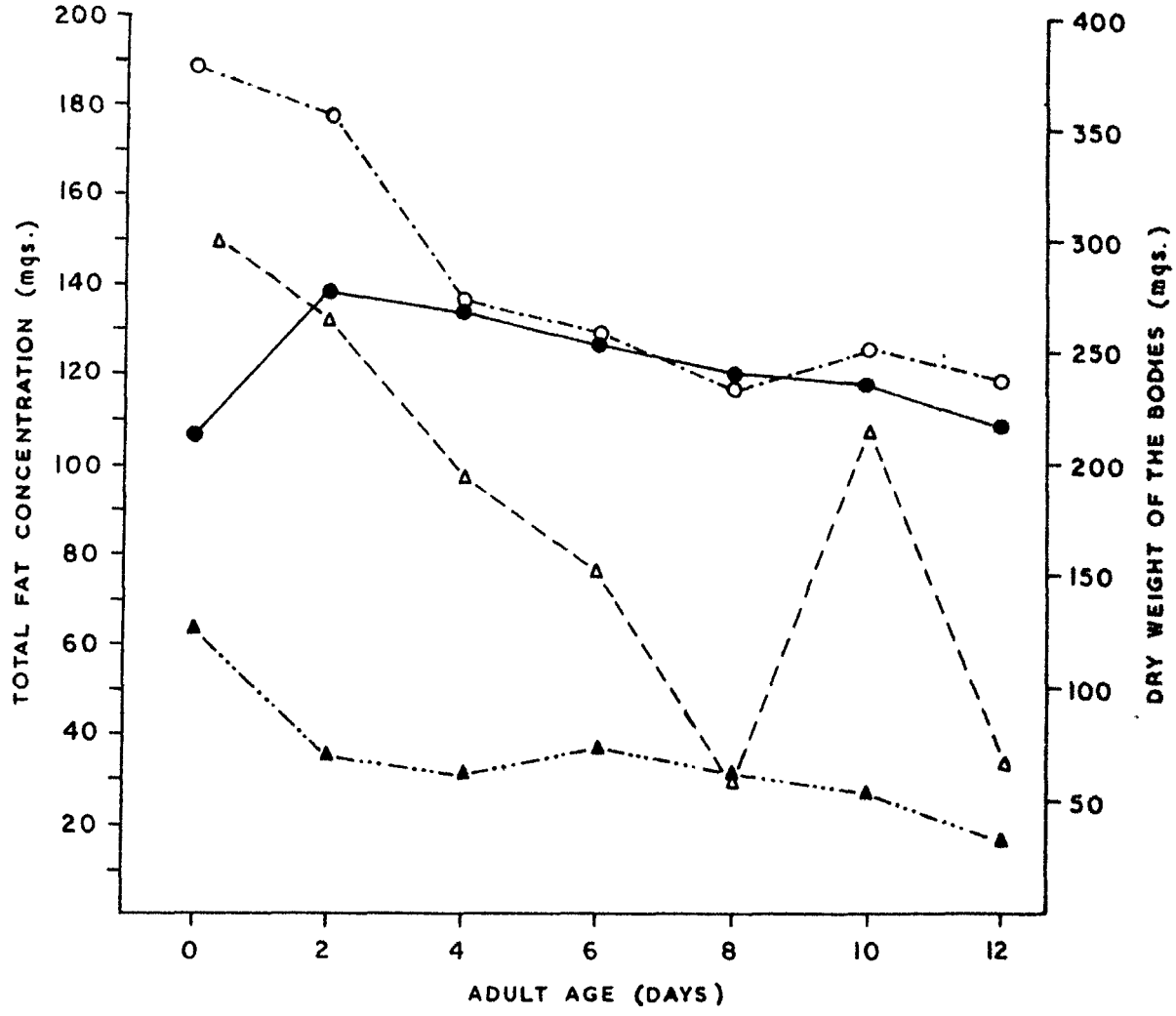

TOTAL FAT CONCENTRATION (mgs.)
DRY WEIGHT OF THE BODIES (mgs.)
ADULT AGE (DAYS)
200
180
160
140
120
100
80
60
40
20
400
350
300
250
200
150
100
50
0
2
4
6
8
10
12

FIG. 19

Fig. 20. Percentage of total fats with regard to dry body weight of the nymphs (4th and 5th) and the adults (both sexes) of D. circulatus related with age.

-o- = percentage of total fat in 4th instar nymphs

-●- = percentage of total fat in 5th instar nymphs

-▲- = percentage of total fat in females

-△- = percentage of total fat in males

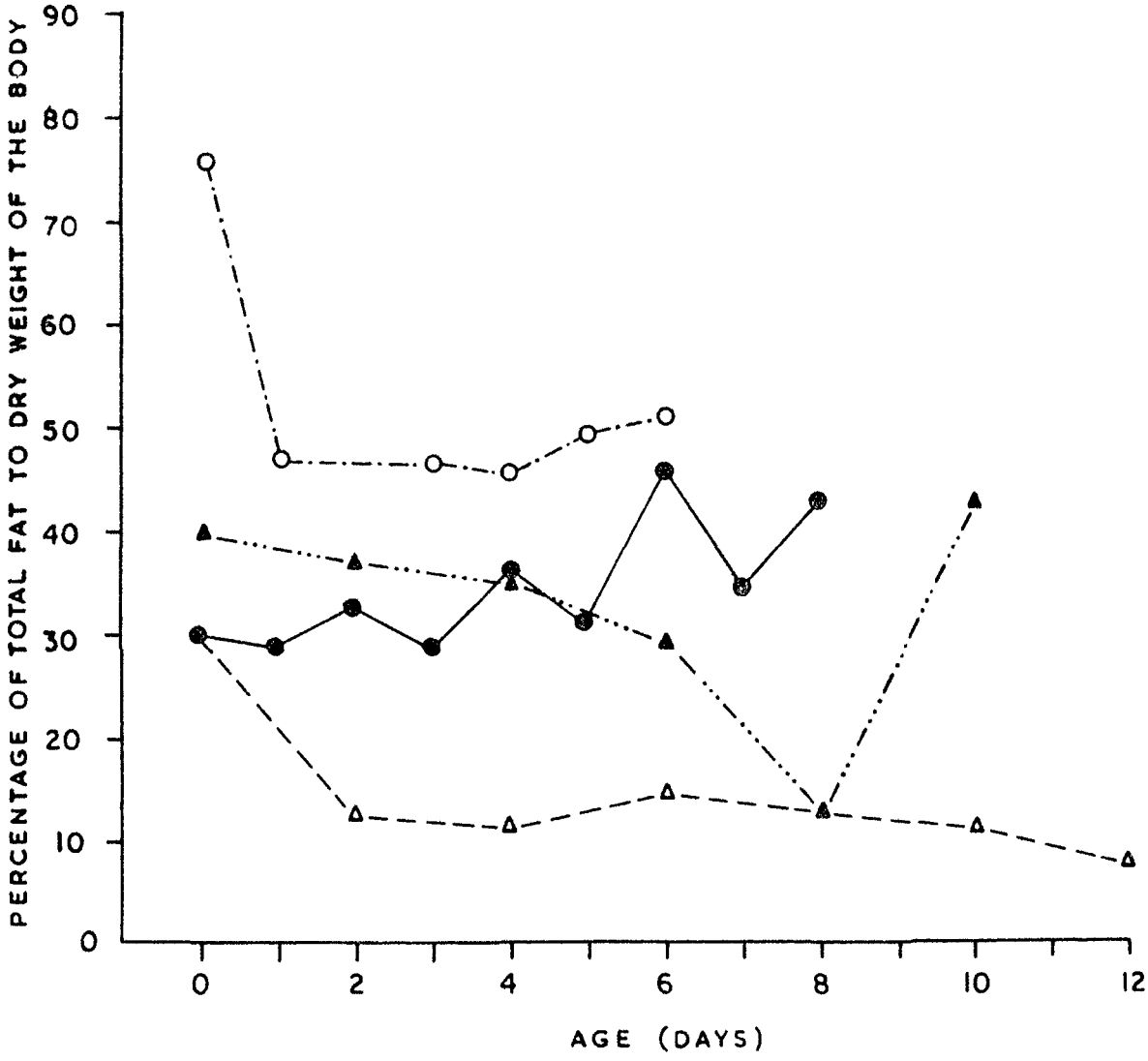

PERCENTAGE OF TOTAL FAT TO DRY WEIGHT OF THE BODY
90
80
70
60
50
40
30
20
10
0
0
2
4
6
8
10
12
AGE (DAYS)

FIG. 20

built up within the next two days which was statistically significant than that of the 8 day old females (t = 10.395). At the same time, the percentage of the fat became 42.595% once again approximating to the initial percentage in the newly emerged females. Again, in the 12 day old females, the TFC as well as the fat percentage dropped (Table 26, Fig. 20). This fall in the total fat concentration was also significant as compared to the TFC of the 10 day old females (t = 6.829).

In contrast to the males, in the females, the dry body weight of the females initially dropped and thereafter fluctuations occurred. However, in the 12 day old females the dry body weight was less than that of the initial weight of the newly emerged females (Fig. 19).

On comparing the percentage of the total fat in the nymphs and the adults of different age, it was observed that the highest peak of the fat percentage with respect to the dry weight per gramme fresh body weight was recorded in the newly moulted 4th instar nymphs (Fig. 20), which decreased rapidly in the beginning i.e. it was reduced to fifty per cent in two days following moulting and gradually increased reaching a second peak of higher percentage of just before the moulting to the 5th instar nymphs. Next, in the newly moulted 5th instar nymphs, the percentage of the fat considerably dropped and it was reduced to more than fifty per cent from that of the maximum percentage of the newly moulted 4th instar nymphs. It

further increased gradually attaining a highest peak of the fat percentage during the nymphal period of the 5th instar, two days before metamorphosis. However, this percentage was never higher than that of the 4th instar nymphs (Fig. 20). Following the metamorphosis, the percentage of the total fat in the newly emerged males was equal to that of the newly moulted 5th instar nymphs but slightly higher in the females, suggesting that the fat accumulated during the 5th instar period goes with the metamorphosed adults and more especially in the females. Thereafter, there was continuous decrease in the fat percentage in both the sexes indicating the utilization of the larval fat by the adults in development and reproduction. In the females, the fluctuations in the fat percentage was related with the reproductive cycle (Fig. 20).

XVII DISCUSSION

From the present study on *D. circulatus*, it is clear that the body weight varies in the nymphal as well as in the adult stage. Further, the changes in the weight are correlated with the morphogenetic condition. There is a rapid increase in the body weight during the first half of each nymphal instar (4th as well as 5th) and later, the addition in the weight is a slow process. Such a process may be related to faster rate of synthesis of the chief metabolites as well as that of development and growth of the tissues. Moreover, rapid synthesis of the raw materials is essential pre-requisite to lay down the new cuticle before moulting. However, before moulting to the next stage, the nymphs attain maximum weight but the shortfall in the body weight either after moulting to next instar or emergence of the adults can be accounted due to the evacuation of the gut for moulting as well as the removal of the old cuticle. It is well known that prior to moulting feeding is stopped and alimentary canal is cleared off its contents. Further, air is sometimes pumped in the gut to make the body distended to facilitate the breaking of the cuticle and eclosion.

The newly emerged males proportionately suffered higher loss of the body weight than the females because the nymphs which were the future females were on the average heavier than

those which would become the males. In the adults, the higher rate of increase in the body weight of the females is a consequence of the growth of voluminous ovaries than small sized testes in case of the males. D. cingulatus is a species in which the ovaries are telotrophic and the growth as well as the maturation of the eggs entirely takes place during the adult life. Hence, the females have to synthesize yolk of the eggs. The ovarian growth is progressive and it is maximal prior to the oviposition of the eggs. Following the oviposition of the first batch of the eggs, remarkable decrease in the ovarian weight as well as that of the whole body is an important consequence of reproduction in this sex, than in the opposite sex. Since D. cingulatus has generally three occasions of oviposition spaced with intervals of a few days during 2 weeks from emergence at the present temperature and humidity, although the males survive much longer than the females, a second set of the oocytes further mature. This is accompanied by another phase of deposition of yolk and maturation leading to again increase in the volume and weight of the ovaries and that of the body as a whole. The oviposition of the second batch of the eggs is again followed by a decrease in the weight of both the ovaries as well as the whole body. Therefore, in the female D. cingulatus there are cyclical changes in the ovarian and the body weight related to the reproductive conditions. In the present study on D. cingulatus, observations have been made only up to twelve days following emergence at the aforesaid

temperature and humidity, and this period covers only two reproductive cycles. The observations could not be continued beyond this period because of natural death and reduction in the population of the females. It is further interesting to record that following the emergence of the females the maturation of the first batch of eggs takes longer time than that of the second batch. It can be pointed out that in the beginning oogenesis and differentiation of the oocytes in the germarium takes sometime before the migration of the oocytes into the vitellarium. Once the mass of the oocytes is differentiated in the germarium, their growth and maturation may not take longer period for their migration in the vitellarium and maturation there. As soon as the first batch of the eggs passes down to the oviduct, the younger oocytes from the germarium move to the vitellarium for maturation.

The rate of increase in the weight of the ovaries and that of the body relsted to the second sexual cycle is significantly lower than that of the first cycle. The difference explains the reduction in the fecundity as the female grows older when the metabolic potential is also lower. This difference is also evident from the higher number in the first batch of the eggs than that of the second batch of this species as examined by Ansari (1971).

The changes in the body weight have been studied in Locusta migratoria (Phipps, 1950 and Chen, 1952). In this

species also, maximum body weight is observed just before the oviposition of the first batch of the eggs. Further, in Schistocerca gregaria and Nomadacris septemfasciata (Norris, 1954, and 1959a) and Drosophila melanogaster (Cummings et al., 1971) ovaries have maximum weight in fully matured condition. Although morphogenetic changes in relation to larval growth and metamorphosis have been recorded in several insect species, the pace of the changes in the body weight of the nymphs of D. cingulatus related with moulting and metamorphosis throws a new light on the biology of this species. The pace of growth in this species can be understood by ten times increase in the body weight of the nymphs undergoing metamorphosis as compared to the newly moulted 4th instar nymphs. In Drosophila species also the larval growth is accompanied with intensive addition of body weight (Chen, 1960).

Parallel with the rapid increase in the body weight of the nymphs during the first half of each instar, there is a change in the total proteins of the body likewise, the proteins also undergo slow synthesis in the later part of the instar. Further, the rate of the protein synthesis in the 5th instar, is more than double of the 4th instar. This accounts for higher protein requirement by the older nymphs. As far as the initial rapid synthesis of the proteins is concerned, during the nymphal instars, it is to produce enough raw material for the building of the new cuticle before the next moult. It is well known that

ecdysone (moulting hormone) as well as juvenile hormone stimulate the protein synthesis during the larval growth (Wigglesworth, 1972). Therefore, it is obvious that higher protein synthesis in the early part of the instar is one of the requisite of the moulting to the next stage. Since form and growth in insects are manifested by the growth of the new cuticle, it is important to know that in D. cingulatus alongwith Pyrrhocoris apterus (Zmmerich, 1970) and Euchenolthus fasciatus (Sister and Drothy, 1966) total proteins of the body must increase as early as possible before moulting to provide enough time for the formation of the new cuticle following the stimulation by the moulting hormone. However, the protein synthesis is continued throughout the instar but it is being continuously utilised until the endocuticle is perfectly formed. Therefore, in the second half of the 4th and the 5th instars of D. cingulatus concentration of the total proteins is weaker in the body. Further, the higher body weight during this period accounts for increase in this metabolite. It can be pointed out that synthesis of the specific proteins related with moulting has been recorded in Rhodnius prolixus (Hamoudan, 1973), Periplaneta americana (Steinhauer and Stephen, 1959 and Fox and Hills, 1969), Locusta migratoria (McCormic and Scott, 1966) and Galleria mellonella (Janda, 1975).

In the fully grown nymphs of D. cingulatus, lower rate of protein synthesis indicate remarkable catabolism of the proteins prior to metamorphosis. This can be interpreted by

the changes occurring during the histolysis of the larval tissues and the histogenesis of the imaginal tissues which involve the degradation of the larval proteins into amino acids which will give rise to adult proteins. Degradation of the larval proteins into amino acids can be supported by the high rate of amino acid nitrogen found in the newly emerged males and females of Schistocerca gregaria (Kulkarni and Mehrotra, 1970).

Total proteins of the body undergo shortfall during the moulting of the larva. This may be also due to the removal of the old cuticle during the moulting. This can be supported by the fact that the moulting fluid may dissolve the proteins of the old cuticle as has been reported by Passmanau and Williams (1953) in Hyalophora species and in Bombyx mori by Zielinska and Laskowska (1958).

In the adult D. cingulatus total proteins of the body undergo remarkable increase in the female than in the male following emergence. But in the females, the changes are related with the maturation of ovaries, increase in the ovarian weight as well as that of the whole body. Therefore, the proteins contribute a significant addition to the body weight. Thus, the proteins concentration in the females is always maximum prior to oviposition and least following the egg laying. It is also elucidative that the changes in the ovarian weight depend on the protein synthesis related with the reproductive

cycle. The increase in the ovarian proteins is either due to endogenous synthesis in the ovarian tissue or through the absorption from the haemolymph. However, transfer of the proteins in the ovaries from the fat body through the haemolymph in relation to the egg maturation has been reported in Pyrrhocoris antaxius (Clâma, 1964), Rhodnius prolixus (Coles, 1965), Leucophaea maderae (Engelmen and Penney, 1966 and Scheurer, 1969), Schistocerca gregaria (Kulkarni and Mehrotra, 1970), Tenebrio molitor (Penrick and Butz, 1970), Triatoma infestans (Perasi, 1973), Musca domestica (Gadallah et al., 1970a) and Glossina austeni (Tobe and Davey, 1975).

Besides, the proteins are also synthesized in the follicle cells and in the preyolk oocytes as observed by Cruickshank (1971) and Imberski and Gertsen (1974) in Ephestia kuhniella as well as in Periplaneta americana (Zinkeister et al., 1971) and in Drosophila melanogaster (Goldstein and Snyder, 1973).

Thus in the female D. cingulatus the rate of the protein synthesis is more rapid and high during the preoviposition period of the first batch of the eggs than that of second reproductive period. This clearly indicates that the protein synthesis as well as its turnover either in the ovary or in the whole body of the female suffers due to ageing. This can also be supported by the fact that D. cingulatus female lays smaller number of eggs in the second batch than in the first.

However, this information on *D. cingulatus* is in contrast to that of *Phormia regina* in which Levenbook and Indira (1971) observed an apparent higher rate of protein synthesis in older flies than that of the young ones. Although, they emphasised on the actual high rate of the protein synthesis in the younger flies.

Since both the body weight as well as the total proteins concentration of the male *D. cingulatus* do not change in the same manner as in the female following emergence, therefore, a pronounced synthesis of the proteins related with mating behaviour of the male is not found to suggest cyclical metabolism of the proteins in the males. It can also be inferred that in the male *D. cingulatus* gonadal proteins are either very poorly synthesized or there is very little absorption of this material from other tissues. The testes of *Drosophila subobscura* (Clark and Maynard, 1966) do not contribute major part of the proteins to the body. Slama (1964) and Emmerich (1970) in *D. antarcta* also concluded that the males do not accumulate haemolymph proteins during its development.

The qualitative study on the separation of various protein bands of the ovaries of *D. cingulatus* in relation to age and reproduction further elucidates and supports the changes in the TPC of the ovaries as described before. From this study it is clear that the number of the proteins in the ovaries soon after emergence is less than that of a couple of day old females.

Further, as the ovaries mature, some of the proteins conjugate and concentrate, thereby their number is reduced but concentration increases. This change is parallel with that of the changes of TPC during the first reproductive period. Further, the repetition of similar changes in the second reproductive period is also in conformity with the quantitative changes in the TPC of the ovaries. Towards the end of the second reproductive period, the number of the ovarian proteins is more reduced than before and qualitatively their concentration is weaker as well. This further signifies that ageing causes lower rate of protein synthesis in the body specially in the yolk of the eggs. Here again there is a point of difference from the observations made in Phormia regina. It appears that the explanation made by Levenbook and Indira (1971) in P. regina that the older flies might have lower rate of turnover of precursor pool for the protein synthesis. It can be pointed out that older insects might show more accumulation of amino acids (precursor pool) due to either catabolic activity or lack of the transformation metabolism of amino acids in to proteins during the old age.

Similar changes in the number of the proteins studied by qualitative method have also been reported in Leucophaea maderae (Dejmal and Brocke, 1968), Nauphoeta cinerea (Adiyodi, 1968) and Hyalophora cecropia (Patel, 1971) related with maturation of the ovaries and age. Wattiaux and Libion (1971) explained that in D. melanogaster, the rate of the protein

synthesis decreases in the older flies because of ageing which involved a progressive loss of stability of the messenger RNA templates responsible for the synthesis of the proteins. Therefore, such a situation is obvious in showing low rate of protein synthesis in other species affected by older age.

A comparative study of the topical application of LD_{50} of sublethal doses of Lindane and Parathion was made on the pattern of ovarian proteins in D. cingulatus with a view to assess the effectivness of such doses of these different insecticides on the protein synthesis related with maturation of the ovaries. The data provided a clear difference between the two insecticides and Lindane (a chlorinated hydrocarbon) was more effective in reducing the number of the proteins during the maturation of the eggs in both the cycles. Further, the concentration of the protein bands also became weaker as compared to that of the normal females. It can be pointed out that whatever quantity of Lindane reached to the ovaries through the haemolymph it suppressed the enzyme activity involved in the protein synthesis in the ovaries. However, an indirect effect on the synthesis of yolk proteins may be through the mode of action of the insecticide on the nervous system. Thompson and Moller (1959) in Calliphora erythrocephala Meign. have proved that the neurosecretory cells of the brain control the protein synthesis in these flies. Since the enzymes are structurally proteins, therefore, the inhibition in the enzyme activity for

protein synthesis is a logical consequence of the mode of action of the insecticides. The synthesis of the protein fractions involved in the vitellogenesis of Musca domestica are strongly inhibited by the action of γ-BHC which alters the ultrastructure of the neurosecretory cells of the protocerebrum (Ramade, 1967 and LeBrun et al., 1973). Further, Esaac et al. (1976) also observed decrease in the haemolymph proteins by the topical application of Endrin (a chloronated hydrocarbon) on Spodoptera littoralis.

On the contrary, Parathion which is an organophosphorus compound, had disintegrating effect on the protein bands in both the reproductive cycles. Further, qualitatively the concentration of the major bands of parathion affected ovaries, undergoes dilution. It appears that the sublethal dose (LD_{50}) of Parathion not only supresses the enzyme activity for protein metabolism but also destroys the structural entity of the various proteins in the ovaries of D. cingulatus. Thus, even the sublethal dose of Parathion may be utilised in suppressing the fecundity of this major pest because of its more effectiveness in the control as compare to that of Lindane on the basis of the present result. It can be further emphasized that the mode of action of an insecticide cannot only be judged by its knocked down property but also its action direct or indirect on the organs of the body especially the reproductive organs which may reduce the population as a result of the action of the chemicals on the gonads. With this view the present comparative results on the

effectiveness of Lindane and Parathion on the ovarian proteins is a significant contribution towards understanding the mode of action of the insecticides.

The changes in the total reducing sugars during the 4th and the 5th instar period of D. cingulatus are almost corresponding to that of the total proteins. This may be ascribed to the rapid synthesis and accumulation of the carbohydrate reserves (glycogen and trehalose) prior to moulting and metamorphosis. Thus, there is turnover of TRS present in various tissues of the body to the metabolites i.e. glycogen in muscles etc. and trehalose in the blood. Both the substances are known as energy giving molecules in insects. As compared to the concentration of the total proteins in the whole body of the nymphs, TRS level is generally lower. It is well known that in the synthesis of chitin, polysaccharides are involved and the availability of free molecules of hexoses and pentoses are rather not possible when the new cuticle is in formation. Incorporation of glucose in the chitin of the epidermis has been recorded in silkworm (Wyatt, 1967). In several insects (Wyatt, 1967 and Wigglesworth, 1972) during metamorphosis, the glycogen reserve of the body tissues as well as trehalose as blood sugar decrease, suggesting glycolysis for the release of the energy required in this process. It is possible that in D. cingulatus the drop in TRS level during the moulting or metamorphosis is a consequence of the oxidation of reducing sugars for obtaining energy to be utilised in ecdysis.

The newly emerged females have lower level of TRS than that of the males, it is possible that this difference is on account of the sexual dimorphism with regard to the body weight, consequently the females may require higher energy for ecdysis. Further, the utilization of the monosaccharides (reducing sugars) may be higher by the developing gonads which are more massive in the females than in the males.

In D. cinculatus following emergence the variation in TRS is also parallel with that of TPC. However, the decrease in TRS of the males is generally due to the fact that glucose and other reducing sugars are utilized in oxidative processes for the motility of the sperms (Geer et al., 1972 and Veera & Rao, 1976). It has been further explained that trehalose being the major blood sugar is hydrolysed to glucose molecules during the maturation of the sperms and later, this monosaccharide is oxidised to provide energy for the motility of the sperms in Eubrachilla annulipes (Veera and Rao, 1976). It can be further supported by the observations of Blum (1962) in drone honey bee, Rao (1967, 1972) in Culex lastularius and Geer et al. (1972) in Drosophila hydei that enzyme trehalase is localised in the seminal fluid and not in the sperms. The functional significance of such an arrangement in insect semen is apparent by the fact that it provides for conservation of large amounts of glucose released by the activity of trehalose to provide energy by oxidation of the glucose at the appropriate time of activation

and motility of the sperms. In Culex lectularius, Rao (1967) has further evidenced on the utilisation of seminal sugars by the motile sperms. However, it is also available that fructolysis takes place in the semen of the honey bee (Blum, 1962) like that of bull semen (Mann, 1940). Thus, both glycolysis as well as fructolysis in the semen of the mature gonad account for a lower TRS level in the body.

In D. cingulatus, the newly emerged male at the present controlled temperature and humidity commence mating from 3rd day following emergence and this is the time when TRS of the male body falls down showing a relationship between the TRS and sexually active males. However, in the older males a slight increase in the TRS may be due to further synthesis of these sugars which may be slowly utilised for sperm motility. Further, in the older males the production of sperms may go down, thereby the glycolysis in the seminal fluid may be reduced. Moreover, the glucose available from the diet or by the breakdown of the glycogen reserves may be more freely available to the body and due to ageing, the synthesis of the enzymes required for the oxidation of the reducing sugars is inhibited (like that of other proteins).

Like the changes in the TPC that of the TRS of the females D. cingulatus is related with the sexual cycle, which is more significant than that of the males. The increased level of the TRS prior to oviposition both in the whole body and in the ovarian

tissue is maximum and following the oviposition minimum. This suggests that reducing sugars are available to the ovaries for the production of the yolk although the source of glucose may be from other tissues of the body (fat body and haemolymph). Here again due to ageing glucose metabolism is reduced as available from the data during the second reproductive cycle of D. sinculatus. The higher concentration of TRS in the preovi-position stages can be justified by the occurrence of the trehalose and the glycogen in the matured ovaries of a number of several species of insects such as Bombyx mori (Zaluska, 1959; Saito, 1963; Yamashita and Hasegawa, 1974), Rhodnius rucina (Orr, 1964), D. melanogaster (Butterworth & Bodenstein, 1968), Anthonomus grandis (Nettles et al., 1971), Nauphoeta cinerea (Barojni & Chakko, 1974) and Hyalophora cecropia (Wyatt, 1974). Since reducing sugars are the intermediate substances in carbohydrate metabolism, therefore, their concentration may be an index to the synthesis of the polysaccharides. Thus, in Periplaneta americana the rapid activation of trehalose synthesis is due to continuous production of glucose (Matthews and Downer, 1974 and Matthews et al., 1976). Hence in D. sinculatus also it can be reasonably inferred that TRS level in the whole body as well as in the ovaries is an index to yolk synthesis (maturation) in the eggs and this material correspondingly goes along with the TPC.

The changes in the total fat concentration (TFC) of the body in the 4th and the 5th instar D. sinculatus are different

than that of the concentration of the total proteins and the reducing sugars. Thus, the metabolism of fat has no parallel with that of the other two substances. Although in the 4th instar soon after moulting the nymphs have maximum lipids in their body, the synthesis of this substance is only apparent after a couple of days. Even such increase in the TFC does not reach to an intermoult peak. On the other hand in the 5th instar there is a progressive increase in the TFC of the body and maximum concentration occurs prior to metamorphosis. However, the bulk of the fat is added during the first half of this final instar indicating a rapid rate of synthesis by the younger larvae of the last instar. The contrast between the 4th and the 5th instars suggests that the lipids are utilized more than synthesis in the 4th instar whereas in the 5th instar there is only making up of a reserve of fat prior to the metamorphosis. It is further evident that in D. cingulatus, the synthesis of the TFC during the second half of the 4th and the 5th instars is partly at the expense of the total reducing sugars because as available from the present data TRS during the corresponding period is depleted. This can be further supported by the fact that inter-conversion of the carbohydrates to lipids is well known during the developmental stages of insects (Gilbert, 1967 and Wigglesworth, 1972).

During the moulting the TFC significantly decreases and thus in the newly moulted 5th instar larvae, the concentration of the total fats carried over from the last instar is about one

third of that of the maximum concentration of the 4th instar (newly moulted). However, in the metamorphosed females the larval fat reserve is less consumed than the males during the metamorphosis. Since fat is the chief source of reserve material and energy, during ecdysis it may release the required energy for the process by its stepwise degradation. It has already been pointed out before that reducing sugars are weak in concentration in the body of the nymphs, therefore, the later may not provide the requisite amount of energy for moulting. Further, the fat reserves of the female nymphs undergoing metamorphosis is essential to be conserved because of its utility in the future for the yolk formation in the eggs. Therefore, higher concentration of the reducing sugars in the female nymphs than the male nymphs is also important because the former have to depend chiefly on the sugars to obtain energy during the moulting. Thus, it can be inferred that there is a clear sexual dimorphism in the last nymphal instar with respect to metabolic activity of the total reducing suggars and lipids prior to metamorphosis. Although this fact has not been directly investigated presently, it will be further confirmed by the future investigations. Utilisation of free lipids to obtain energy for moulting has been reported in Tenebrio molitor (Horan, 1959), Bombyx species (Niemierko, 1959), Tribolium confusum (Villeneuve and Lemonde, 1963) and Lygus plasmicollis (Mauldin, et al., 1971).

Following emergence, the TFC in both sexes of *D. cingulatus* suffers adversely because of the consumption of the larval fat either as a source of energy to the individuals or by its degradation to simpler substances which may be utilized elsewhere in the body such as the tissue building etc. Such a state of affair can be supported by the fact that in the same species the adipohaemocytes gradually reduced in number following emergence in both sexes and especially significantly in the females (Zaidi and Khan, 1975). However, in the females, the significant drop in the TFC following oviposition is obvious due to the removal of a substantial amount of lipids of the yolk in ripe eggs. But, before oviposition of the second batch of the eggs there is some increase in the TFC which may be in response to the demand of the maturing oocytes for the next batch of the eggs. It is further interesting that during the first reproductive cycle of the females the metabolism of the reducing sugars is related with the TFC decrease. The latter may provide higher concentration of the former by its degradation. Although larval fat reserve undergoes breakdown with the advancement of the age, the fresh synthesis of the lipids during the second reproductive cycle may be a consequence of the nutritional intake and metabolism as well as the inter-conversion of the hydrolysed products of other metabolites (proteins and carbohydrates).

However, in the males the metabolic requirement does not correspond to that of the females. This is clear from the fact

that TFC undergoes only reduction with the advancement of age and there is no further synthesis of fat. Therefore, in the males the fat may either be utilised in obtaining energy throughout life or the breakdown material is used for the tissue building and maturation of the sperms which is a continuous process.

From the present investigation on D. cingulatus it is generally concluded that the behaviour and the pattern of the synthesis and utilisation of the total proteins, total reducing sugars and lipids vary during the developmental stages and growth of the adults. However, the changes in the metabolism of the first two substances are not parallel with that of the last substance. Further, female D. cingulatus differs from that of the male with regards to clear changes in the metabolism of these substances related with the reproductive activity of the female. It is important to mention that present data on the metabolic changes of the proteins, the reducing sugars and the fats are fundamental contribution of our existing knowledge among Hemiptera especially on D. cingulatus. Moreover, the metabolic changes in different proteins of the ovaries by the effect of the insecticides used presently contribute to the mode of action of the insecticides on the ovarian protein. This information may be further used in the assessment of the effectiveness of different insecticides in controlling the fecundity and the fertility of D. cingulatus.

XVIII SUMMARY

1. In Dysdercus cingulatus Fabr. (Hemiptera:Pyrrhocoridae) the concentration of the total proteins (TPC), reducing sugars (TRS) and fats (TFC) has been quantitatively determined in the whole body of the developing nymphs (4th and 5th instars) related with age and moulting as well as in the adults of both sexes from emergence to twelve post-emergent days which include two sexual cycles.

2. Besides, the concentration of the total proteins and the reducing sugars was also recorded in the ovaries of the females of different age in relation to their reproductive cycles.

3. Furthermore, the ovarian proteins of the normal as well as affected females by the topical application of LD_{50} of Parathion and Lindane on the last instar nymphs of D. cingulatus were separated on the Polyacrylamide gel to observe changes on the synthesis of the ovarian proteins.

4. The body weight of the growing nymphs of the 4th as well as 5th instar increases with age and there is a rapid as well as significant rise in the early period of the instars.

5. However, moulting of the nymphs to the next stage is correlated with significant fall in the body weight.

6. Prior to the last moulting i.e. metamorphosis, the nymphs become ten times heavier than those of the newly moulted 4th instar.

7. The body weight of the males is significantly lower than that of the females at the time of emergence and it does not significantly increase with the advancing age.

8. On the other hand, in the females, the body weight is maximum before oviposition and soon after it falls down.

9. The changes in the ovarian weight are related with the maturation of the eggs and these are also parallel with that of the body weight.

10. The significantly higher weight of the ovaries in the 7 day old females(following the oviposition) as compared to that of the newly emerged females indicates that the growth of the ovaries and maturation of the eggs are completely an imaginal process.

11. The changes in the protein synthesis of the whole body during the nymphal stage of D. cingulatus are parallel with that of the body weight.

12. The rate of the protein synthesis is rapid in the early part of each instar and slows down in the later part.

13. The final instar nymphs (5th instar) synthesize proteins more than double of the 4th instar, showing the need of the protein reserve prior to metamorphosis.

14. There is a short fall in the total proteins concentration of the body (TPC) after the moulting or the metamorphosis due to the removal of the old cuticle.

15. In both the instars, corresponding to the changes in the TPC, the level of total reducing sugars (TRS) also initially rises and depletes prior to the moulting or metamorphosis suggesting a rapid turnover of these sugars into the polysaccharides (glycogen and trehalose). However, the fully grown nymphs have higher concentration of reducing sugars than that of the previous stage.

16. The turnover of the TRS into carbohydrate reserve during the second half of each instar maintains the TRS level inversely proportional to the body weight.

17. There is an insignificant drop in the TRS concentration after the moulting or metamorphosis as a consequence of the oxidation of some of the reducing sugars for the energy requirement during ecdysis.

18. During the 4th instar, the newly moulted nymphs have maximum concentration of total fat (TFC) in their body and later the level is kept below.

19. But in the 5th instar (final instar) there is progressive increase in the TFC in the older nymphs. Maximum TFC occurs before metamorphosis. Thus bulk of larval lipids are synthesised in the last nymphal instar.

20. The TPC markedly drops during moulting of the nymphs and emergence of the adults due to the consumption of the fat to release energy for ecdysis.

21. In the adult D. cingulatus, the changes in the TPC, TRS and TFC are different in the two sexes.

22. The TPC in the whole body of the newly emerged males and the females does not significantly differ. However, in the females the variations are correlated with the reproductive cycles i.e. a rise in the maturing eggs and decrease after oviposition.

23. In the males, the TPC remains almost constant throughout during the twelve post-emergent days except small but insignificant increase in the older males.

24. Further, the changes in the TPC of the females are parallel with that of the body weight as well as ovarian weight indicating higher protein synthesis related with the development and maturation of the eggs.

25. The newly emerged females have lower concentration of the TRS than that of the males of the corresponding age.

26. In the male D. cingulatus an initial increase in the TRS level is a pre-requisite for obtaining energy for the sperm motility by its oxidation.

27. In the females, the changes in the concentration of the reducing sugars are parallel with that of the total proteins

concentration and the body weight.

28. The changes in the ovarian weight and its TPC and TRS levels also run parallel with that of the weight of the females and their protein concentrations. This confirms that the synthesis of the protein and carbohydrate increases in the ovaries with the maturation of the eggs.

29. But the changes in the body lipids of the adults are completely different than those of the total proteins and the reducing sugars.

30. The larval fat reserve is more consumed by the males than by the females during the metamorphosis, clearly indicating a sexual dimorphism with regard to the physiology of the metamorphosis in this species. Further, the nymphs destined to emerge as the females conserve their fat for the yolk formation during the maturation of the eggs.

31. Following the emergence, the body lipids decrease considerably in both the sexes, indicating their continuous utilisation during the growth and the reproduction of the adults.

32. But in the males, the fall in the lipid content is less pronounced and within 12 post-emergent days only 1/4th of the total fat of the newly emerged males is consumed chiefly as a source of energy for the vital activities as well as in the synthesis of other metabolites e.g. proteins and carbohydrates in old age.

33. Conversely, in the females, during the first reproductive cycle, there is more significant loss of the TPC following the oviposition suggesting that most of the larval fat is utilized in the maturation of the first batch of the eggs.

34. Further, fresh synthesis of the lipids was required for the formation of the yolk in the maturing eggs of the next batches.

35. The qualitative study of different soluble proteins of the the ovaries confirms the fact that the TPC in the ovaries increases with the maturation of the eggs, because maximum number of the protein fractions are detected preceding maturation of the ovaries.

36. Further, it is also clear that related with the maturation of the eggs, concentration of certain proteins qualitatively increases.

37. Following the oviposition of the first batch of the eggs, appearance of certain minor bands of ovarian proteins raises the total number of the proteins, suggesting fresh synthesis of those proteins prior to the maturation of the next batch of the eggs.

38. The electrophoretic study of the ovarian proteins shows that anodic proteins are more involved in the reproductive cycles as compared to that of the cathodic proteins.

39. The topical application of the sublethal dose (LD_{50}) of Lindane and Parathion results in the supression of the synthesis of the ovarian proteins. Thus in the lindane affected females the number of the ovarian proteins is reduced.

40. However, Parathion (organophosphorus compound) is comparatively more effective because it not only reduces the concentration of different proteins but also disintegrates the major protein fractions of the affected ovaries.

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APPENDIX X TABLES

APPENDIX TABLE 1. 4th INSTAR : TOTAL BODY WEIGHT (mgs)

No. of individual	AGE (DAYS)								
	ZERO	1	2	3	4	5	6	7	8
1	11.0	14.0	20.0	22.0	27.0	25.4	28.4		
2	9.5	10.5	21.4	25.0	26.6	27.0	29.0		
3	9.5	14.0	19.8	24.4	24.0	27.0	30.0		
4	13.5	14.4	25.0	27.4	26.4	24.8	32.0		
5	11.5	11.5	22.4	24.0	28.6	26.5	37.0		
6	8.5	12.0	22.5	25.0	27.4	23.0	29.8		
7	8.0	10.4	20.5	28.5	25.5	27.0	30.0		
8	9.0	8.5	22.0	24.5	28.0	29.0	27.0		
9	7.8	8.8	24.0	20.0	25.0	24.0	26.0		
10	14.0	10.8	20.5	22.0	26.0	26.0	20.0		
11	5.5	7.0	23.0	27.0	27.5	27.0	29.0		
12	6.0	12.0	24.0	25.0	25.0	31.0	28.5		
13	5.5	6.0	22.0	25.5	26.4	25.0	30.0		
14	8.5	10.0	21.0	26.4	29.0	30.0	27.0		
15	5.5	9.6	20.4	30.0	30.0	28.8	22.0		
16	8.4	9.0	18.5	25.0	27.5	28.0	27.0		
17	10.5	8.0	20.0	22.0	27.0	26.0	29.0		
18	9.4	14.5	22.0	27.0	25.0	27.0	35.0		
19	7.4	6.0	22.5	27.5	26.5	27.0	31.0		
20	8.5	5.0	20.0	22.0	26.0	26.0	26.5		
MEAN (mg)	8.875	10.10	21.575	25.01	26.72	27.025	29.11		
	5th INSTAR								
1	18.5	30.0	40.8	45.0	56.5	68.5	74.4	80.5	90.0
2	26.6	28.6	38.5	50.4	70.5	70.4	75.4	68.4	95.5
3	23.2	30.5	40.2	55.0	68.5	70.5	68.4	75.2	96.8
4	28.0	25.4	43.0	45.0	59.0	75.2	79.5	75.5	80.4
5	29.0	25.5	34.4	50.0	70.0	68.3	82.0	80.0	88.2
6	22.0	28.5	35.5	55.0	66.4	70.0	69.4	89.2	76.0
7	24.5	30.0	45.5	55.5	68.5	60.5	80.0	74.4	68.0
8	25.2	30.5	40.2	58.0	75.0	66.0	63.4	73.4	74.5
9	27.0	26.0	40.5	40.0	70.0	78.0	75.0	88.0	86.4
10	20.5	30.0	42.0	55.5	74.2	67.5	78.5	90.5	95.5
11	24.5	24.0	48.0	46.0	65.0	74.0	70.5	70.5	95.0
12	20.0	18.0	38.0	46.8	66.0	76.0	73.2	72.5	95.0
13	22.0	28.0	43.0	56.0	62.0	69.0	70.5	80.0	80.0
14	22.5	30.0	48.5	47.5	70.2	60.0	70.0	85.5	86.4
15	18.0	21.0	35.0	51.4	60.5	68.0	68.0	74.4	93.0
16	21.0	14.5	35.5	48.0	65.4	72.0	74.4	75.0	80.0
17	23.0	29.5	37.0	55.0	70.6	70.2	75.5	67.5	92.5
18	24.0	25.0	40.0	51.5	64.0	62.0	69.0	78.0	78.0
19	25.0	26.5	30.5	44.0	67.0	65.4	74.0	74.0	82.4
20	25.4	20.0	40.0	50.0	60.0	65.0	73.6	70.0	80.0
MEAN (mg)	23.495	26.075	39.805	50.255	66.465	68.825	73.485	77.125	85.680

APPENDIX TABLE - 2. ADULT: SEX: MALE - TOTAL BODY WEIGHT

No. of individuals	AGE (DAYS												
	ZERO	1	2	3	4	5	6	7	8	9	10	11	12
1	36.5	42.3	40.0	31.0	32.0	30.0	32.0	28.0	25.0	30.0	45.0	32.0	45.0
2	40.0	30.0	45.0	34.0	30.0	25.0	30.7	28.0	28.0	27.0	40.0	34.0	48.0
3	42.3	42.0	35.0	40.0	25.6	35.0	45.0	45.0	35.0	35.0	35.0	50.0	35.0
4	30.0	40.0	30.0	35.0	40.1	35.0	40.0	35.0	35.0	30.0	35.0	40.0	40.0
5	42.0	35.0	45.0	32.0	32.0	37.0	40.0	28.0	28.0	45.0	35.0	45.0	30.0
6	35.0	40.0	44.0	35.0	28.0	44.0	35.0	35.0	35.0	40.0	40.0	40.0	30.0
7	40.0	30.0	30.0	40.0	42.0	45.0	40.0	30.0	42.0	45.0	49.0	52.0	44.0
8	45.0	28.0	30.0	45.0	45.0	40.0	45.0	35.0	35.0	45.0	45.0	49.0	49.0
9	30.0	40.0	25.0	30.0	25.0	35.0	30.0	35.0	28.0	28.0	40.0	40.0	40.0
10	32.0	45.0	28.0	38.0	25.4	30.0	40.0	40.0	35.0	30.0	35.0	45.0	35.4
11	35.0	35.0	35.0	32.0	30.0	36.0	40.0	25.0	40.0	35.0	45.0	34.0	40.2
12	35.0	30.0	40.0	35.0	35.0	40.0	39.0	30.0	42.0	40.0	30.0	35.4	40.0
13	30.4	43.0	35.0	34.2	30.0	41.0	25.0	25.0	44.2	35.0	39.0	40.0	39.6
14	32.0	33.0	42.0	28.0	30.0	34.5	28.0	28.0	30.0	40.0	45.0	44.0	38.0
15	28.6	35.0	36.0	40.0	38.0	40.0	41.0	32.0	35.0	34.2	40.0	36.5	35.0
16	35.0	38.0	38.0	35.5	48.5	42.0	32.5	29.4	41.0	30.0	48.0	48.4	40.0
17	36.8	32.0	34.0	44.0	32.0	30.0	30.0	30.0	30.0	30.0	36.0	44.0	40.0
18	32.2	35.0	28.0	38.0	40.0	33.0	37.0	35.0	34.0	33.0	34.0	40.0	47.0
19	38.0	26.0	40.0	36.0	34.0	36.4	38.0	28.0	30.0	32.0	40.0	37.2	39.0
20	35.4	35.0	30.0	30.4	33.8	35.0	30.0	30.0	35.0	29.5	32.5	35.0	45.0
21	35.0	30.0	36.5	41.0	32.0	30.0	33.0	38.0	35.5	35.0	30.0	35.0	40.0
22	36.5	40.0	35.0	38.0	28.0	32.0	38.0	35.0	34.0	35.4	40.0	42.0	36.5
MEAN (mg)	35.577	35.650	35.522	36.004	33.472	35.722	35.463	32.018	34.395	34.731	39.022	40.840	39.850

FEMALE

1	75.2	76.0	67.6	63.0	56.0	90.8	90.0	92.0	125.0	83.4	137.0	125.0	53.0
2	66.2	78.0	61.8	68.0	42.2	90.0	115.1	120.0	80.0	120.0	141.0	98.0	79.0
3	68.0	62.0	60.0	70.0	87.0	100.0	92.0	82.0	60.0	76.0	75.0	90.0	94.0
4	61.0	70.0	69.0	62.0	90.0	55.0	100.0	110.0	65.0	70.0	100.0	120.0	130.0
5	60.0	60.0	60.0	65.0	40.0	45.0	86.0	115.0	117.0	75.2	107.0	75.0	75.0
6	65.0	70.0	70.0	74.0	54.0	41.0	118.0	95.0	80.0	78.4	100.0	65.0	105.0
7	62.0	62.0	60.0	103.0	66.0	95.0	117.0	95.0	115.0	100.0	97.4	80.0	95.0
8	70.0	65.0	70.2	103.0	68.0	130.0	114.0	83.0	41.0	115.0	106.0	100.0	127.0
9	74.8	67.0	55.0	75.0	86.0	117.0	100.0	113.0	110.0	57.0	80.0	95.0	75.0
10	68.2	74.0	62.0	65.0	76.0	80.0	113.0	85.0	55.0	110.0	85.0	115.0	130.0
11	55.0	60.0	57.0	70.0	62.0	65.0	116.0	130.0	85.0	62.0	80.0	115.0	82.0
12	50.0	58.0	60.0	65.0	80.0	80.0	120.0	62.0	53.0	112.0	120.0	120.0	132.0
13	49.0	70.0	64.0	54.0	95.0	60.0	115.0	75.0	60.0	65.0	82.0	120.0	132.0
14	60.0	75.0	80.0	62.0	80.0	102.0	103.0	93.0	80.0	110.0	115.0	100.0	75.0
15	70.4	65.0	80.0	60.0	65.0	106.0	150.0	80.0	125.0	122.0	72.0	122.0	99.0
16	72.0	72.0	60.0	75.0	68.0	98.0	130.0	74.2	82.0	113.0	95.0	110.0	115.0
17	70.0	69.0	70.0	70.0	86.0	108.0	126.0	90.0	75.0	110.0	115.0	80.0	125.0
18	63.0	72.5	55.0	69.0	76.0	110.0	140.0	75.0	85.0	83.0	130.0	85.0	75.0
19	55.0	62.4	65.0	66.2	80.0	100.0	90.0	79.0	104.0	100.0	100.0	130.0	82.0
20	71.2	63.0	70.0	75.0	105.5	95.0	130.0	75.0	88.0	120.0	92.0	102.0	90.4
21	60.0	70.0	64.0	71.0	90.0	97.0	105.0	110.0	80.0	95.0	107.0	90.5	104.5
22	69.0	65.0	80.0	75.0	80.0	105.0	100.5	78.0	100.0	112.0	100.0	125.0	78.0
23	62.0	65.0	71.5	68.5	80.5	89.0	102.0	85.0	75.0	100.0	105.0	100.0	100.0
24	60.0	67.0	67.0	66.8	97.0	140.0	112.0	115.0	95.0	85.0	135.0	94.4	75.0
MEAN (mg)	64.041	67.412	65.795	70.645	75.425	91.616	111.886	92.133	84.375	94.750	103.183	102.37	96.912

APPENDIX TABLE 3 . SEX : FEMALE - OVARY: DRY WEIGHT (mg)

No. of individuals	AGE (DAYS)												
	ZERO	1	2	3	4	5	6	7	8	9	10	11	12
1	6	7	5	20	15	22	55	8	15	30	40	44	12
2	4	8	7	15	25	55	70	15	13	41	20	48	19
3	6	6	8	20	15	35	40	15	55	20	22	25	10
4	5	8	16	35	20	40	45	15	13	15	35	45	18
5	12	9	15	25	40	40	50	15	23	16	34	44	43
6	6	15	10	20	35	35	33	10	45	30	33	16	15
7	4	5	5	25	15	32	46	5	40	19	25	20	3
8	4	15	5	22	40	22	35	6	35	50	30	40	5
9	7	3	8	20	46	28	70	13	30	27	35	45	5
10	18	15	16	25	20	40	51	15	35	35	45	50	4
MEAN (mgs)	7.2	9.1	8.5	22.7	27.1	34.9	49.5	11.7	28.6	28.3	31.9	37.7	13.4
						FRESH WEIGHT							
1	9	10	16	28.8	33.8	40.5	51.0	28.6	45.0	40	38.0	22	21
2	14	9.5	19.5	25.5	29.5	42.2	48.8	13.8	39.2	51.5	32.0	20.5	32.2
3	10	15	19.4	31.2	40.0	49.0	65.6	19.4	38.8	41.0	30.2	61.2	19
4	11.4	14	15	20	25.0	53.0	53.0	25.0	43.4	31.0	56.2	25	25.5
5	8.2	11.2	11	30.5	36.6	32.0	60.0	18.0	23.8	48.0	62.0	24	27
6	19.5	10.0	22	29	35.0	25.0	53.20	15.4	27.4	29.0	59.0	20.2	25
7	12.0	9.2	12	33	44.0	35.0	59.0	22.2	19.2	30.4	48.0	52	18.10
8	10	13.0	18	27	27.9	40.0	42.4	28.8	29.5	34.0	49.0	27	23
9	18	8	13.2	31.2	48.4	37.0	69.0	20.5	34.0	32.0	31.2	60	22
10	16	12	10	30	32.2	30.0	54.4	17.4	30.0	26.0	37.0	22	16
MEAN (mg)	12.81	11.19	15.61	28.62	35.24	38.37	55.64	20.91	33.03	36.29	44.25	33.39	22.88

APPENDIX TABLE 4. 4th INSTAR NYMPHS : TOTAL PROTEIN CONCENTRATION

No. of individuals	AGE (DAYS)										
	ZERO	1	2	3	4	5	6	7	8	9	10
1	.0181	.0285	.0282	.0331	.0320	.0301	.0387				
2	.0210	.0123	.0186	.0306	.0338	.0344	.0298				
3	.0136	.0214	.0234	.0292	.0360	.0407	.0366				
4	.0222	.0208	.0266	.0312	.0303	.0334	.0446				
5	.0113	.0200	.0236	.0318	.0407	.0326	.0432				
6	.0235	.0157	.0235	.0352	.0302	.0416	.0424				
7	.0125	.0192	.0129	.0303	.0431	.0333	.0388				
8	.0144	.0117	.0256	.0353	.0571	.0401	.0418				
9	.0166	.0147	.0277	.0382	.0306	.0345	.0280				
10	.0285	.0120	.0258	.0377	.0319	.0307	.0487				
MEAN (mg)	.0169	.0174	.0236	.0331	.03657	.03514	.03926				

	5th INSTAR NYMPHS									Newly moulted adult Female	Newly moulted adult Male
1	.0216	.0243	.0294	.0355	.0486	.0401	.0551	.0658	.0588	.0526	.0554
2	.0195	.0252	.0285	.0350	.0531	.0426	.0530	.0409	.0502	.0575	.0519
3	.0243	.0370	.0231	.0363	.0496	.0375	.0497	.0505	.0650	.0587	.0460
4	.03089	.0222	.0255	.0355	.0474	.0498	.0503	.0635	.0597	.0507	.0532
5	.0298	.0222	.0212	.0340	.0478	.0490	.0500	.0681	.0617	.0449	.0461
6	.0211	.0326	.0318	.0411	.0451	.0535	.0504	.0538	.0631	.0536	.0514
7	.0163	0.208	.0300	.0401	.0474	.0438	.0475	.0470	.0558	.0468	.0576
8	.0224	.0305	.0314	.0431	.0500	.0424	.0497	.0449	.0550	.0452	.0543
9	.0307	.0280	.0229	.0425	.0478	.0480	.0506	.0545	.0625	.0452	.0513
10	.0146	.0243	.0221	.0408	.0505	.0503	.0445	.0530	.0659	.0525	.0519
MEAN (mg)	.02511	.02731	.02659	.03839	.04873	.0457	.0500	.05420	.05977	.05075	.05191

APPENDIX TABLE - 5. TOTAL PROTEIN CONCENTRATION - MALE

No. of individuals	AGE (DAYS)												
	ZERO	1	2	3	4	5	6	7	8	9	10	11	12
1	0.0420	0.0300	0.0450	0.0400	0.0321	0.0500	0.0321	0.0510	0.0572	0.0566	0.0606	0.0593	0.0628
2	0.0680	0.0340	0.0520	0.0360	0.0500	0.0406	0.0770	0.0642	0.0642	0.0579	0.0591	0.0588	0.0610
3	0.0640	0.0470	0.0680	0.0470	0.0742	0.0590	0.0644	0.0606	0.0571	0.0542	0.0590	0.0546	0.0514
4	0.0340	0.0470	0.0650	0.0590	0.0357	0.0618	0.0566	0.0514	0.0542	0.0600	0.0568	0.0516	0.0688
5	0.0830	0.0640	0.0370	0.0730	0.0633	0.0675	0.0682	0.0839	0.0642	0.0606	0.0675	0.0670	0.0755
6	0.0670	0.0990	0.0340	0.0700	0.0642	0.0620	0.0514	0.0457	0.0428	0.0591	0.0682	0.0591	0.0821
7	0.0450	0.0470	0.0630	0.0470	0.0357	0.0444	0.0500	0.0600	0.0452	0.0441	0.0462	0.0519	0.0340
8	0.0440	0.0400	0.0940	0.0520	0.0377	0.0450	0.0422	0.0457	0.0514	0.0422	0.0322	0.0489	0.0482
9	0.0470	0.0590	0.0460	0.0370	0.0412	0.0524	0.0600	0.0485	0.0642	0.0607	0.0716	0.0716	0.0500
10	0.0480	0.0620	0.0460	0.0470	0.0405	0.0533	0.0475	0.0475	0.0590	0.0566	0.0647	0.0670	0.0508
11	0.0440	0.0540	0.0540	0.0450	0.0500	0.0550	0.0475	0.0412	0.0375	0.0571	0.0628	0.0558	010563
12	0.0700	0.0470	0.0590	0.0510	0.0514	0.0546	0.0600	0.0476	0.0333	0.0591	0.0476	0.0564	0.0500
MEAN (mg)	0.0540	0.0527	0.0554	0.0500	0.0480	0.0530	0.0540	0.0538	0.0520	0.0554	0.0570	0.0585	0.0575

FEMALE

1	0.0390	0.0440	0.0340	0.0523	0.0232	0.0436	0.0510	0.0391	0.0376	0.0360	0.0386	0.0392	0.0269
2	0.0410	0.0450	0.0630	0.0448	0.0378	0.0627	0.0478	0.0375	0.0387	0.0352	0.0386	0.0397	0.0357
3	0.0580	0.0540	0.0680	0.0607	0.0471	0.0410	0.0690	0.0603	0.0333	0.0598	0.0606	0.0616	0.0601
4	0.0600	0.0570	0.0530	0.0798	0.0611	0.0327	0.0685	0.0544	0.0369	0.0607	0.0495	0.0591	0.0600
5	0.0470	0.0500	0.0590	0.0730	0.0750	0.0688	0.0500	0.0678	0.0508	0.0578	0.0742	0.0670	0.0740
6	0.0600	0.0620	0.0600	0.0574	0.0629	0.0731	0.0694	0.0578	0.0750	0.0650	0.0710	0.0952	0.0604
7	0.0590	0.0480	0.0500	0.0531	0.0566	0.0447	0.0679	0.0447	0.0491	0.0425	0.0816	0.0243	0.0594
8	0.0600	0.0540	0.0620	0.0533	0.0477	0.0488	0.0557	0.0578	0.0487	0.0491	0.0773	0.0425	0.0468
9	0.0370	0.0500	0.0410	0.0733	0.0325	0.0846	0.0850	0.0805	0.0772	0.0753	0.0387	0.0631	0.0573
10	0.0360	0.0580	0.0550	0.0476	0.0184	0.0425	0.0805	0.0964	0.0920	0.0327	0.0505	0.0713	0.0823
11	0.0400	0.0560	0.0580	0.0785	0.0822	0.0553	0.0706	0.0761	0.0800	0.0903	0.0675	0.0713	0.0439
12	0.0230	0.0480	0.0580	0.0384	0.0800	0.0675	0.0725	0.0887	0.0641	0.0758	0.0758	0.0733	0.0840
13	0.0870	0.0610	0.0480	0.0555	0.0894	0.0900	0.0860	0.0800	0.0633	0.0830	0.0658	0.0891	0.0856
14	0.0560	0.0640	0.0770	0.0483	0.0887	0.0976	0.0963	0.0333	0.0562	0.0772	0.0739	0.0880	0.0480
MEAN (mg)	0.0505	0.0536	0.0561	0.0582	0.0573	0.0609	0.0692	0.0624	0.0573	0.0772	0.0616	0.0631	0.0588

APPENDIX TABLE 6. SEX : FEMALE - OVARY : TOTAL PROTEIN CONCENTRATION

No. of individual sample	AGE (DAYS)												
	Zero	1	2	3	4	5	6	7	8	9	10	11	12
1	.0166	.0180	.020	.0120	.0266	.0195	.0236	.0125	.0266	.0173	.0166	.0295	.0300
2	.0162	.0125	.021	.0200	.0260	.0150	.0228	.0153	.0153	.0153	.0200	.0172	.0210
3	.0166	.0166	.012	.0120	.0220	.0123	.0257	.0153	.0138	.0200	.0347	.0200	.0230
4	.0130	.0162	.008	.0113	.0250	.0200	.0192	.0153	.0176	.0243	.0228	.0184	.0200
5	.0083	.0144	.020	.0120	.0209	.0207	.0260	.0243	.0158	.0206	.0243	.0219	.0154
6	.0166	.0153	.013	.0200	.0143	.0142	.0251	.0130	.0095	.0143	.0151	.0250	.0153
7	.0162	.0130	.026	.0260	.0153	.0250	.0318	.0200	.0107	.0263	.0172	.0182	.0100
8	.0162	.0153	.020	.0227	.0212	.0320	.0237	.0166	.0207	.0166	.0166	.0207	.0130
9	.0142	.0216	.016	.0270	.0253	.0300	.0228	.0155	.0221	.0148	.0190	.0214	.0060
10	.0166	.0153	.014	.200	.0215	.0207	.0287	.0133	.0120	.0190	.0147	.0213	.0070
MEAN (mg)	.0150	.0158	.0170	.0183	.0217	.0209	.0249	.0161	.0164	.0188	.0201	.0213	.01607

APPENDIX TABLE 7 : 4th INSTAR LARVA TRS/mg BODY WEIGHT

No. of individual	AGE (DAYS)								
	ZERO	1	2	3	4	5	6	7	8
1	.00109	.00180	.00217	.00214	.00174	.000162	.00110		
2	.00233	.00169	.00225	.00168	.00176	.00187	.00105		
3	.00145	.00125	.00181	.00172	.00174	.00136	.00113		
4	.00188	.00215	.00190	.00204	.00172	.00180	.00096		
5	.00109	.00218	.00176	.00260	.00186	.00180	.00054		
6	.00190	.00133	.00183	.00192	.00174	.00171	.00096		
7	.00152	.00150	.00170	.00154	.00170	.00153	.00110		
8	.00170	.00234	.00200	.00200	.00168	.00170	.00114		
9	.00189	.00226	.00168	.00210	.00173	.00162	.00109		
10	.00188	.00215	.00190	.00163	.00169	.00169	.00105		
MEAN (mg)	.00167	.00186	.00190	.00193	.00173	.00167	.00101		

5th INSTAR LARVA

1	.00089	.00141	.00204	.00195	.00267	.00232	.00181	.00175	.00183
2	.00100	.00088	.00189	.00273	.00287	.00252	.00229	.00176	.00149
3	.00090	.00157	.00190	.00282	.00248	.00278	.00236	.00202	.00147
4	.00097	.00180	.00226	.00265	.00279	.00173	.00248	.00203	.00097
5	.00066	.00133	.00154	.00260	.00221	.00226	.00170	.00196	.00135
6	.00085	.00096	.00135	.00187	.00293	.00247	.00231	.00194	.00110
7	.00095	.00162	.00145	.00283	.00286	.00182	.00241	.00174	.00149
8	.00091	.00136	.00175	.00275	.00278	.00167	.00168	.00192	.00107
9	.00096	.00150	.00118	.00195	.00286	.00232	.00235	.00200	.00133
10	.00102	.00130	.00165	.00200	.00260	.00227	.00220	.00131	.00170
MEAN (mg)	.00091	.00137	.00170	.00241	.00270	.00221	.00215	.00184	.00137

APPENDIX TABLE 8. ADULT MALE - TRS/mg BODY WEIGHT

No. of individuals	AGE (DAYS)												
	ZERO	1	2	3	4	5	6	7	8	9	10	11	12
1.	.00105	.00125	.00137	.00128	.00100	.00097	.00088	.00080	.00099	.00057	.00097	.00110	.00095
2.	.00168	.00109	.00142	.00085	.00106	.00127	.00078	.00085	.00073	.00105	.00120	.00109	.00089
3.	.00062	.00108	.00133	.00165	.00142	.00095	.00107	.00118	.00114	.00064	.00110	.00098	.00080
4.	.00108	.00126	.00142	.00135	.00173	.00071	.00098	.00074	.00107	.00060	.00137	.00115	.00115
5.	.00125	.00131	.00135	.00177	.00112	.00106	.00073	.00086	.00073	.00066	.00105	.00118	.00095
6.	.00074	.00132	.00142	.00121	.00155	.00096	.00113	.00125	.00111	.00084	.00094	.00090	.00110
7.	.00163	.00076	.00140	.00161	.00135	.00115	.00142	.00078	.00066	.00068	.00105	.00096	.00082
8.	.00124	.00102	.00093	.00105	.00112	.00085	.00066	.00080	.00080	.00061	.00086	.00188	.00111
9.	.00160	.00073	.00120	.00170	.00087	.00073	.00084	.00110	.00107	.00068	.00060	.00188	.00110
10.	.00115	.00120	.00131	.00163	.00064	.00093	.00110	.00102	.00052	.00090	.00100	.00109	.00126
MEAN (mg)	.00121	.00110	.00131	.00141	.00119	.00096	.00093	.00095	.00088	.00073	.00103	.00124	.00103
	FEMALE												
1.	.00099	.00098	.00115	.00073	.00113	.00149	.00128	.00107	.00087	.00113	.00086	.00103	.00078
2.	.00133	.00125	.00073	.00149	.00152	.00102	.00121	.00095	.00067	.00106	.00107	.00094	.00090
3.	.00100	.00121	.00082	.00111	.00120	.00146	.00145	.00048	.00113	.00101	.00123	.00067	.00107
4.	.00088	.00132	.00069	.00086	.00121	.00165	.00125	.00089	.00077	.00069	.00140	.00072	.00050
5.	.00069	.00086	.00073	.00114	.00150	.00122	.00153	.00100	.00081	.00100	.00104	.00109	.00092
6.	.00109	.00120	.00100	.00109	.00149	.00117	.00158	.00106	.00072	.00116	.00108	.00088	.00068
7.	.00073	.00125	.00071	.00101	.00135	.00121	.00150	.00112	.00121	.00094	.00130	.00070	.00084
8.	.00095	.00089	.00140	.00117	.00122	.00133	.00139	.00106	.00094	.00108	.00124	.00105	.00051
9.	.00090	.00092	.00106	.00078	.00129	.00112	.00154	.00072	.00105	.00098	.00133	.00094	.00082
10.	.00080	.00104	.00086	.00080	.00144	.00157	.00142	.00117	.00121	.00077	.00142	.00080	.00056
MEAN (mg)	.00095	.00110	.00092	.00102	.00130	.00130	.00141	.00097	.00094	.00099	.00119	.00088	.00071

APPENDIX TABLE 9. OVARY - TRS/mg OVARIAN TISSUE

No. of individuals ovary	AGE (DAYS)												
	ZERO	1	2	3	4	5	6	7	8	9	10	11	12
1	.00044	.00060	.00062	.00104	.00082	.00148	.00105	.00118	.00120	.00110	.00121	.00045	.00057
2	.00100	.00042	.00143	.00070	.00047	.00080	.00110	.00043	.00102	.00151	.00137	.00048	.00118
3	.00060	.00120	.00051	.00141	.00110	.00106	.00131	.00123	.00118	.00131	.00066	.00120	.00031
4	.00070	.00071	.00040	.00030	.00072	.00116	.00139	.00080	.00110	.00090	.00096	.00072	.00047
5	.00048	.00055	.00036	.00118	.00087	.00112	.00133	.00055	.00109	.00129	.00122	.00075	.00066
6	.00102	.00040	.00154	.00131	.00097	.00072	.00075	.00051	.00051	.00062	.00101	.00059	.00056
7	.00083	.00065	.00033	.00127	.00104	.00114	.00132	.00063	.00052	.00065	.00116	.00065	.00053
8	.00040	.00090	.00055	.00074	.00043	.00090	.00119	.00125	.00061	.00094	.00126	.00051	.00043
9	.00077	.00050	.00045	.00089	.00115	.00086	.00147	.00048	.00094	.00106	.00096	.00100	.00045
10	.00062	.00066	.00040	.00113	.00068	.00093	.00121	.00068	.00106	.00076	.00145	.00072	.00025
MEAN (mg)	.00062	.00066	.00066	.00099	.00083	.00102	.00121	.00078	.00092	.00104	.00112	.00072	.00054

APPENDIX TABLE 10. 4th INSTAR NYMPH : TOTAL FAT CONCENTRATION

No. of observations	AGE (DAYS)								
	ZERO	1	2	3	4	5	6	7	8
1	167	142	111	108	112	107	188		
2	181	123	121	100	156	119	103		
3	179	111	117	144	122	147	94		
4	156	135	105	156	131	156	106		
5	187	95	108	124	97	122	170		
MEAN (mg)	174	121.2	112.4	126.4	123.6	130.2	132.2		

5th INSTAR NYMPH

1	100	77	97	120	139	110.4	161	137.2	176
2	69	71	111	109	168	130	128	107	155
3	74	35	125	97	131	97	144	122	171
4	56.2	55	100	73.5	114	117	130	85	165
5	70.8	47	74	82.5	104	70.6	92	119.8	153
MEAN (mg)	74	57.0	101.4	94.4	131.2	103	131	114.2	164

APPENDIX TABLE 11. MALE : TOTAL FAT CONCENTRATION

No. of observations	AGE (DAYS)						
	ZERO	2	4	6	8	10	12
1	63	35	30	38	21	44	7
2	37	38	29	40	30	20	15
3	55	45	31	51	29	30	31
4	72	31	35	24	16	26	17
5	88	28	29	34	59	15	11
MEAN (mg)	65	35.4	30.8	37.4	31	27	16.2
	FEMALE						
1	172	151	85	62.2	55.6	122	44
2	160	131	75	80.8	33	117	64
3	142	142	109	100.0	19.2	100	30
4	131	121	99	76.0	12	90	18
5	145	116	117	61.0	26.2	106	16
MEAN (mg)	150	132.2	97	76	29.2	107	34.4